# ESTIMATION OF SERUM VASPIN LEVELS IN HUMANS WITH OBESITY AS A NOVEL CIRCULATING AND THERAPEUTIC BIOMARKER OF OBESITY AND ITS RELATED METABOLIC ALTERATIONS

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**Branch V**


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**Madras Medical College and Rajiv Gandhi Government General Hospital**

**CHENNAI –600003**

**THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY**

**CHENNAI –600032**

**MAY 2018**

## CERTIFICATE

This is to certify that the dissertation entitled **"ESTIMATION OF THE SERUM VASPIN LEVELS IN HUMANS WITH OBESITY AS A NOVEL CIRCULATING AND THERAPEUTIC BIOMARKER FOR OBESITY AND ITS RELATED METABOLIC ALTERATIONS." by Dr.THAMIZH VALLI. D,** for M.D Physiology is a bonafide record of the research done by her during the period of the study (2015-2018) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai- 600 003.

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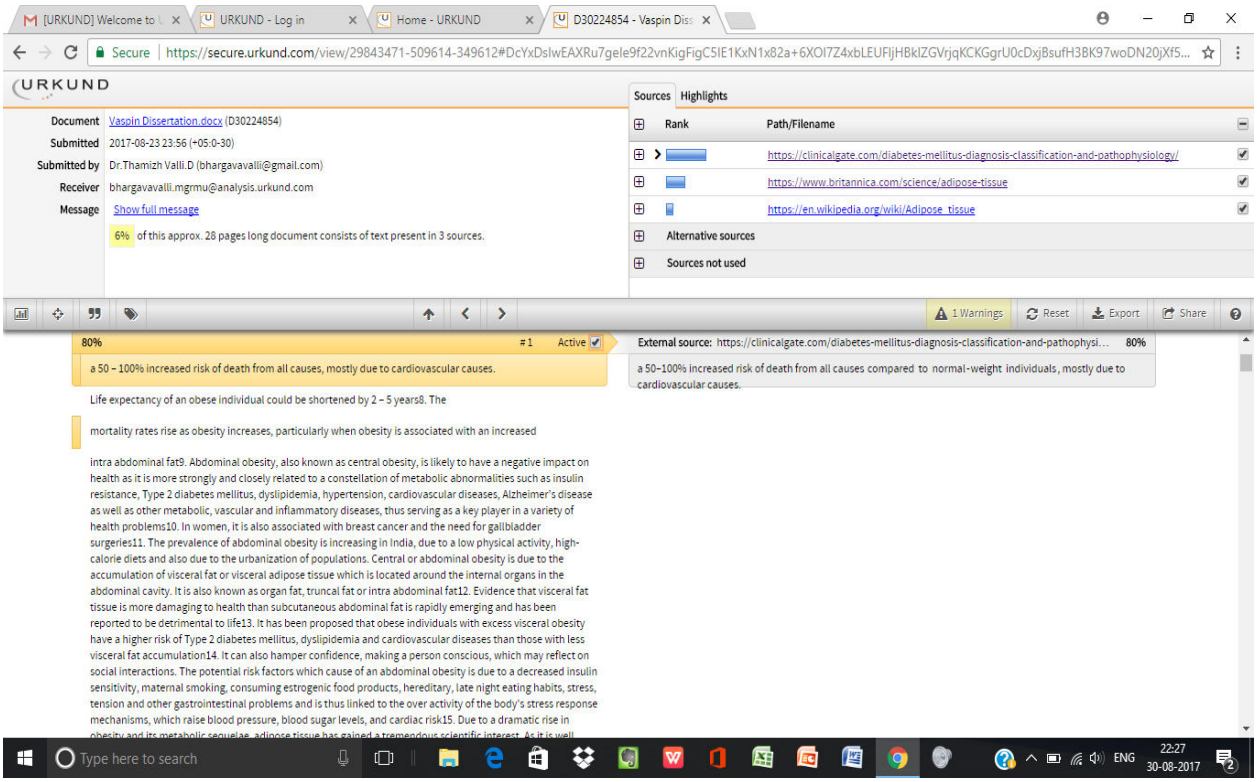
## ABBREVIATIONS

1. BMI – Body Mass Index
2. ELISA – Enzyme Linked Immunosorbent Assay
3. IR – Insulin Resistance
4. DM – Diabetes Mellitus
5. CVD – Cardio vascular disease
6. SVF – Stromal Vascular fraction
7. TNF α – Tumour necrosis factor alpha
8. CRP – C Reactive protein
9. SAA – Serum Amyloid A protein
10. PAI – Plasminogen activator inhibitor
11. MCR 4 – Melanocortin 4 receptor
12. POMC – Pro opio melanocortin
13. ACTH – Adrenocortico trophic hormone
14. PI 3K – Phoshoinositol 3 kinase
15. ras – MAPK – ras mitogen activated protein kinase
16. AOD -1 / SREBP -1 c – Adipocyte determination and differentiation factor – 1/ sterolregulatory element binding protein 1c
17. IRS 1 & 2 – Insulin receptor substrates 1 & 2
18. WAT – White adipose tissue
19. BAT – Brown adipose tissue
20. GLUT 4 – Glucose transporter 4
21. FFA – Free fatty acids
22. IL -6 – Interleukin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a 50 – 100% increased risk of death from all causes, mostly due to cardiovascular causes.
Life expectancy of an obese individual could be shortened by 2 – 5 years8. The
mortality rates rise as obesity increases, particularly when obesity is associated with an increased
intra abdominal fat9. Abdominal obesity, also known as central obesity, is likely to have a negative impact on health as it is more strongly and closely related to a constellation of metabolic abnormalities such as insulin resistance, Type 2 diabetes mellitus, dyslipidemia, hypertension, cardiovascular diseases, Alzheimer's disease as well as other metabolic, vascular and inflammatory diseases, thus serving as a key player in a variety of health problems10. In women, it is also associated with breast cancer and the need for gallbladder surgeries11. The prevalence of abdominal obesity is increasing in India, due to a low physical activity, high-calorie diets and also due to the urbanization of populations. Central or abdominal obesity is due to the accumulation of visceral fat or visceral adipose tissue which is located around the internal organs in the abdominal cavity. It is also known as organ fat, truncal fat or intra abdominal fat12. Evidence that visceral fat tissue is more damaging to health than subcutaneous abdominal fat is rapidly emerging and has been reported to be detrimental to life13. It has been proposed that obese individuals with excess visceral obesity have a higher risk of Type 2 diabetes mellitus, dyslipidemia and cardiovascular diseases than those with less visceral fat accumulation14. It can also hamper confidence, making a person conscious, which may reflect on social interactions. The potential risk factors which cause of an abdominal obesity is due to a decreased insulin sensitivity, maternal smoking, consuming estrogenic food products, hereditary, late night eating habits, stress, tension and other gastrointestinal problems and is thus linked to the over activity of the body's stress response mechanisms, which raise blood pressure, blood sugar levels, and cardiac risk15. Due to a dramatic rise in
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a 50–100% increased risk of death from all causes compared to normal-weight individuals, mostly due to cardiovascular causes.
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## CERTIFICATE - II

This is to certify that this dissertation work titled **"ESTIMATION OF THE SERUM VASPIN LEVELS IN HUMANS WITH OBESITY AS A NOVEL CIRCULATING AND THERAPEUTIC BIOMARKER FOR OBESITY AND ITS RELATED METABOLIC ALTERATIONS "** of the candidate **Dr.THAMIZH VALLI.D** with registration Number **201515004** for the award of **M.D** in the branch of **PHYSIOLOGY** . I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **6 percentage** of plagiarism in the dissertation.

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# ABSTRACT

## ESTIMATION OF SERUM VASPIN LEVELS IN HUMANS WITH OBESITY AS A NOVEL CIRCULATING AND THERAPEUTIC BIOMARKER OF OBESITY AND ITS RELATED METABOLIC ALTERATIONS


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Year : 2018


**BACKGROUND:**

Obesity is associated with metabolic complications and significantly increases the risk of developing insulin resistance, which in turn contributes to the development of Type 2 DM, hypertension, atherosclerosis, coronary heart disease, stroke, metabolic syndrome, and several types of cancer and is thus associated with an increased risk of premature death.

Visceral fat or visceral adipose tissue, is referred to as 'active fat which is potentially dangerous as it is the major player in the adverse metabolic consequences of obesity.

In this context, one of the recently discovered and interesting adipokine that provides a new insight about the physiology, pathology and treatment of obesity is Vaspin.

**Vaspin** is a visceral adipose tissue derived serine protease inhibitor with insulin sensitizing effects, belonging to the serpin superfamily, clade A (Serpin A12).

In humans, vaspin is found to be expressed in the visceral adipose tissues in the stomach, liver and pancreas and also from the skin ( subcutaneous fat) and the hypothalamus. But a significantly higher expression was found from the visceral adipose tissues when compared to the subcutaneous adipose tissues.

An increased vaspin secretion may be due to a compensatory response in order to antagonize the action of other unknown proteases that are up-regulated in obesity and in states of insulin resistance, hence this up-regulation may be a defensive and a protective mechanism aimed to reduce insulin resistance in humans and this protective mechanism of vaspin is lost with the progression to Type 2 DM and the development of microvascular complications.

## AIM OF THE STUDY:

To determine the circulating Serum Vaspin levels in humans with obesity in order to assess its association and link to obesity related metabolic alterations.

**OBJECTIVES**:

1. To estimate the circulating Serum Vaspin levels in humans with obesity and in healthy control subjects.

2. To estimate the anthropometric measurements (i.e the standing height & weight), the measures of obesity (i.e the Waist and Hip Circumference, the Waist/Hip ratio and the BMI) in the humans with obesity and in the healthy control subjects.

3. To estimate the Lipid profile, the Fasting Blood Glucose levels, the Fasting serum insulin levels and the Insulin resistance by the HOMA-IR method in the humans with obesity and in the healthy control subjects.

4. To assess and compare the Serum Vaspin levels and its correlation with the above said parameters in the humans with obesity and in the healthy control subjects.

**MATERIALS & METHODS:**

It is a cross sectional study consisting of thirty obese subjects in the age group of 30 to 55 years having a BMI of $\geq 35$ (Group I) and another thirty subjects of the same age group with a normal range BMI (Group II).

The BMI, the measures of obesity, the fasting blood glucose levels, the lipid profile, the fasting serum insulin levels were obtained. The insulin resistance was estimated by the Homeostasis model assessment method ( HOMA – IR). Serum vaspin levels were

assayed using the commercially available human vaspin ELISA kit using a human vaspin sandwich ELISA technique for both the study groups.

**RESULTS:**

The obese subjects (Group I ) showed significant differences in the BMI, measures of obesity, lipid profile, serum insulin levels, insulin resistance and the serum vaspin levels . ($p<0.001$). Pearson's correlation revealed that serum vaspin levels were positively associated with the age, BMI, waist circumference, hip circumference HDL, LDL, TGL, TC/HDL ratio, LDL/HDL ratio, the fasting blood sugar levels, serum insulin levels and insulin resistance.

**CONCLUSION:**

From this study it can be demonstrated that vaspin may be used as a circulating biomarker for early identification of obesity related metabolic alterations and vaspin also plays an important role in the pathogenesis of obesity and its related metabolic disorders.

**KEY WORDS:**

Obesity, Diabetes mellitus, Vaspin, Insulin resistance.

# INTRODUCTION

# INTRODUCTION

***"LET FOOD BE THY MEDICINE AND MEDICINE BE THY FOOD"***

- ***Hippocrates***

Obesity is a chronic, multifactorial disease involving environmental, genetic, physiologic, metabolic, behavioural, and psychological components. It has been increasing at an alarming rate throughout the world to the extent that it is now a pandemic, affecting millions of people globally[1].

It has become the second leading and a preventable cause of death worldwide, with increasing rates in adults, especially in women and children[2 & 38].

The increasing prevalence of medically significant obesity raises a great concern with an unequivocal trend for further increase.

An increase in the obesity prevalence rate is evident in Westernized countries, where obesity has been present for decades, but today it is also particularly noticeable in the developing countries that previously had not experienced problems with overweight and obesity[3].

Globally, the prevalence of obesity is estimated to be 36.9% for men and from 38.0% for women[4]. There have been substantial increases in the prevalence of obesity in the developing countries as well, which is estimated to be 23.8% for men and 22.6% for women[5].

India is now following a trend of other developing countries that are slowly and steadily becoming more obese. Obesity in India has reached epidemic proportions in the 21st century, with morbid obesity affecting 5% of the country's population[6].

Recent concern is focused on child and adolescent obesity, which is a rapidly growing problem in many countries.

Obesity implies an excess storage of fat to an extent that it may have a negative impact on health[37].

The serious impact of obesity on individuals and societies throughout the world in terms of health, social and economic costs is the major concern.

Obesity has been linked to a host of illnesses such as insulin resistance, Type 2 diabetes mellitus, hypertension, dyslipidemia, metabolic syndrome, cardiovascular diseases, liver diseases, breathing disorders, osteoarthritis, reproductive disorders and certain types of cancer[7].

These complications are caused directly due to obesity or indirectly through mechanisms sharing a common cause.

Insulin resistance plays a crucial role in the pathogenesis of all these disorders and there are many postulated mechanisms of how an insulin resistance develops in obese individual [40, 41]. Obesity increases the risk of physical as well as the mental conditions and as a result contributes to a 50 – 100% increased risk of death from all causes, mostly due to cardiovascular causes. Life expectancy of an obese individual could be shortened by 2 – 5 years[8].

The mortality rates rise as obesity increases, particularly when obesity is associated with an increased intra abdominal fat[9].

Abdominal obesity, also known as central obesity, is likely to have a negative impact on health as it is more strongly and closely related to a constellation of metabolic abnormalities such as insulin resistance, Type 2 diabetes mellitus, dyslipidemia, hypertension, cardiovascular diseases, Alzheimer's disease as well as other metabolic, vascular and inflammatory diseases, thus serving as a key player in a variety of health problems[10].

In women, it is also associated with breast cancer and the need for gallbladder surgeries[11].

The prevalence of abdominal obesity is increasing in India, due to a low physical activity, high-calorie diets and also due to the urbanization of populations.

Central or abdominal obesity is due to the accumulation of visceral fat or visceral adipose tissue which is located around the internal organs in the abdominal cavity.

It is also known as organ fat, truncal fat or intra abdominal fat[12]. Evidence that visceral fat tissue is more damaging to health than subcutaneous abdominal fat is rapidly emerging and has been reported to be detrimental to life[13]. It has been proposed that obese individuals with excess visceral obesity have a higher risk of Type 2 diabetes mellitus, dyslipidemia and cardiovascular diseases than those with less visceral fat accumulation[14].

It can also hamper confidence, making a person conscious, which may reflect on social interactions.

The potential risk factors which cause of an abdominal obesity is due to a decreased insulin sensitivity, maternal smoking, consuming estrogenic food products, hereditary, late night eating habits, stress, tension and other gastrointestinal problems and is thus linked to the over activity of the body's stress response mechanisms, which raise blood pressure, blood sugar levels, and cardiac risk[15].

Due to a dramatic rise in obesity and its metabolic sequelae, adipose tissue has gained a tremendous scientific interest. As it is well known that the adipose tissue is an active endocrine organ which serves largely as a depot for the storage of fat[16].

Apart from this important function, the adipocytes are involved in the energy metabolism and are the source of hormones, cytokines, and metabolites that play an important role in whole-body metabolism and insulin resistance[17].

These cytokines or the bioactive mediators also called the cell signalling proteins secreted by the visceral adipose tissue are known as adipokines or adipocytokines.

These adipokines send signals to organs of metabolic importance including brain, liver, skeletal muscle, and the immune system—thereby regulating the blood pressure, homeostasis, lipid and glucose metabolism, inflammation, hemostasis, angiogenesis and atherosclerosis[18].

Currently there are about 600 known adipokines[19]. Some of the potential adipokines that are involved in the link between obesity and its related metabolic abnormalities, especially insulin resistance are TNF- α, IL -6, adiponectin, retinol binding protein, PAI - 1 and leptin[20].

The novel and the recently discovered adipokines that are also involved in the pathophysiology of obesity and its related metabolic disorders are vaspin, visfatin, resistin, omentin, chemerin etc., [21].

Obesity is strongly associated with alterations in the physiological functions of adipose tissue, leading to insulin resistance, chronic inflammation, and altered secretion of adipokines[22]. An excessive accumulation of fat can cause a dysregulation of the function of the adipocytes thus causing an over secretion of the deleterious adipokines and a hyposecretion of the advantageous ones [23].

An adipose tissue dysfunction or adisopathy plays a crucial role in the different obesity-linked diseases including inflammation, insulin resistance and cancer [24].

The adipose tissue that is diseased and does not function properly is called as sick fat or adisopathy, which results in endocrine and immune responses that would cause metabolic abnormalities and directly promote cardiovascular disease [25].

The harmful effects of visceral fat are due to lipotoxicity. Unlike subcutaneous fat, visceral fat cells release their metabolic products directly into the portal circulation, which is in turn carried to the liver. These visceral fat cells that are enlarged with an excess of triglycerides, pour the free fatty acids into the liver causing them to accumulate in the pancreas, heart and other vital organs. These free fatty acids accumulate in cells, in various locations of the body resulting in an organ dysfunction, which produces impaired regulation of insulin, blood sugar, and cholesterol, as well as abnormal heart functions[26].

Faced with these risks, it's no wonder that one would want to know how much he or she should weigh. But this common and important question is actually the wrong question.

For health, the issue is not how much you weigh, but how much abdominal fat you have. What matters is not the body weight, but the body fat.

The various measures of obesity include the estimation of the body mass index (BMI), waist & hip circumference, waist hip ratio, percentage of body fat and the skin fold thickness[27].

However the waist circumference is globally used as a parameter and is a simplest way to quantify central obesity. This is because it correlates well with excessive visceral fat, which appears to be the most metabolically active fat, which is responsible in causing insulin resistance. Therefore it is considered to be pro-atherogenic[28].

The Index of central obesity is the waist to hip ratio and is a better substitute to the waist circumference in defining central obesity[29].

Measures of central obesity help refine the clinical evaluation of obesity-related risk.

In the late 1980s and early 1990s insightful and powerful imaging techniques that would further help advance the understanding of the health risks associated with body fat accumulation, were discovered[30]. Techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are the most accurate and have made it possible to categorize mass of adipose tissue located at the abdominal level into intra-abdominal fat and subcutaneous fat[31].

Thus a complete assessment of the risks related to adiposity appears to be as important as many other elements of clinical practice and is the first step toward initiating behavioural changes leading to weight loss.

One of the newly discovered adipocytokine is Vaspin (Visceral adipose tissue derived Serpin which was found to have insulin sensitizing effects. It is a member of serine protease inhibitor (SERPIN) family which was first isolated from visceral adipose tissue of Otsuka Long–Evans Tokushima Fatty (OLETF) rats, a model of abdominal obesity and type 2 diabetes[32].

In the diet induced obese OLETF rats, serum vaspin levels were found to be very high at the age when obesity and the plasma insulin levels reached a peak and the administration of vaspin to these obese rats was found to significantly improve their glucose tolerance and insulin sensitivity[33].

Expression of vaspin gene in visceral adipose tissue of humans and an increased circulating levels in the serum was found be positively associated with parameters of obesity, obesity related diseases, insulin resistance, and glucose metabolism [34].

It is also indicated that vaspin plays a role in the adipoinsular axis and is associated with insulin resistance in obese subjects[35].

A significant correlation of vaspin with the adipokine leptin also suggests that the serum vaspin concentrations reflect the body fat mass in humans[36].

Thus my study aims at estimating the serum vaspin levels in obese subjects and investigating the role of vaspin as a biomarker for insulin resistance and the obesity related metabolic alterations, by analysing the correlation between the circulating levels of serum vaspin in humans and the markers of insulin sensitivity, glucose and lipid metabolism.

This study will focus on the regulation of insulin responsiveness by the adipokines and on evidence supporting the hypothesis that these adipokines play a role in the pathophysiology of insulin resistance.

It will also focus upon and review the potential mechanisms by which the visceral adipose tissue depots are responsible in causing an insulin resistance and the other metabolic complications of obesity.

## CAUSES OF OBESITY :

Undoubtedly genes influence the susceptibility to obesity in response to specific diets and availability of nutrition.

Cultural factors are also important – these relate to both availability and composition of the diet and to changes in the level of physical activity[39].

Obesity is attributed to a lot of behavioural features and environmental factors that affect diet and physical activity patterns and also there are many other secondary causes and factors which cause obesity[42].

It has been established that a complex gene environment interaction determines the individual risk to develop obesity[43].

Many other factors such as education and socio economic status may also act as strong modifiers of body weight. Modern lifestyle has caused the trend towards obesity to reach a plateau, which supports the concept that genetic and biologic factors contribute substantially to the susceptibility to develop obesity [44].

Despite the genetic predisposition it is widely accepted that the current worldwide epidemic of obesity is largely a consequence of dramatic changes in lifestyle and environment which emerged over the past 30- 50 years[45].

A dramatic change in eating habits and food selection took place, whereas physical activity decreased remarkably because of technological development concerning transportation and workplaces. A rather novel phenomenon is the expansion of fast food

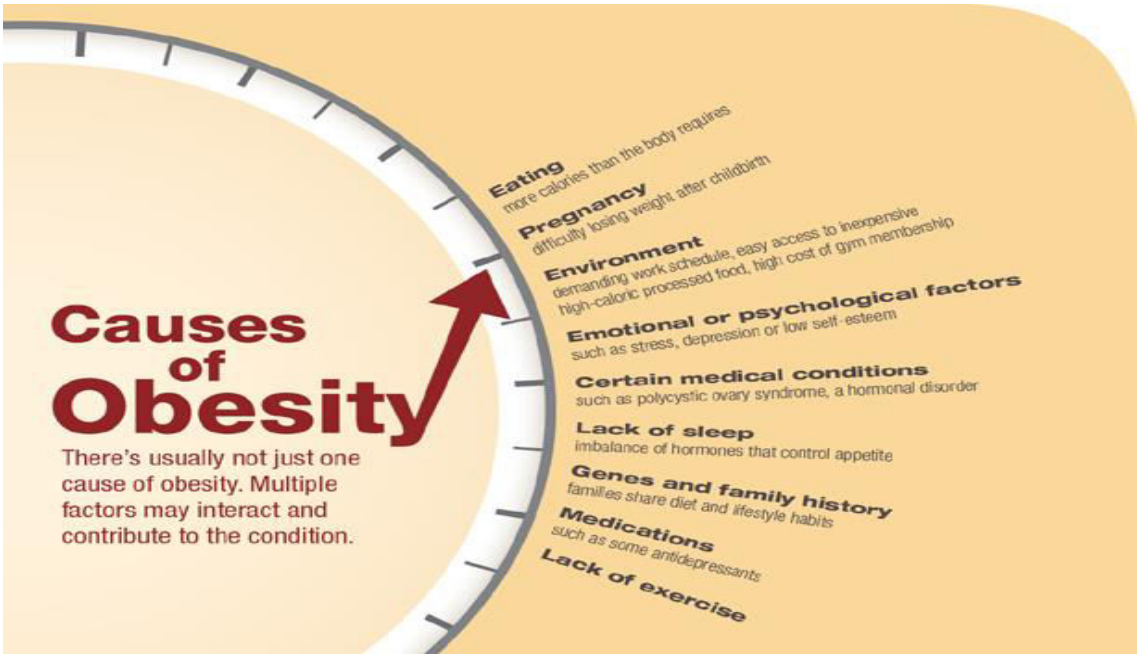
culture characterized by high fat, low starch foods together with a high intake of sugar sweetened beverages which has in turn led to a body weight gain and maintenance of overweight and obesity in the population[46].

The secondary causes include polycystic ovarian syndrome, hypothyroidism, Cushing's syndrome, hypothalamic disease and drug induced weight gain[47].

The common medications include anti diabetic agents, steroid hormones, and psychotropic agents, mood stabilisers, anti depressants and anti epileptic drugs[48].

Another interesting clinical observation is that an excessive weight gain during pregnancy, independent of the initial BMI may also increase the risk of early development of obesity in the offspring and thus possibly increasing the risk for other long term health related consequences[49].

**Figure 1.Causes of obesity :**



## SUSCEPTIBILITY TO OBESITY :

Susceptibility to obesity and its adverse consequences undoubtedly varies between individuals. Twin and adoption studies confirm a genetic influence on obesity.

The pattern of inheritance suggests a polygenic disorder, with small contributions from a number of different genes, together accounting for 25–70% of variation in weight.

Recent results from 'genome-wide' association studies of polymorphisms in large numbers of people have identified a handful of genes that influence obesity, some of which encode proteins known to be involved in the control of appetite or metabolism and some of which have unknown function. However, these genes account for less than 5% of the variation in body weight.

A few rare single-gene disorders have been identified that lead to severe childhood obesity. These include:

- Mutations of the melanocortin-4 receptor (MC4R)
- Defects in the enzymes processing propiomelanocortin (POMC, the precursor for adrenocorticotrophic hormone (ACTH) in the hypothalamus
- Mutations in the leptin gene.

The genetic conditions associated with obesity include:

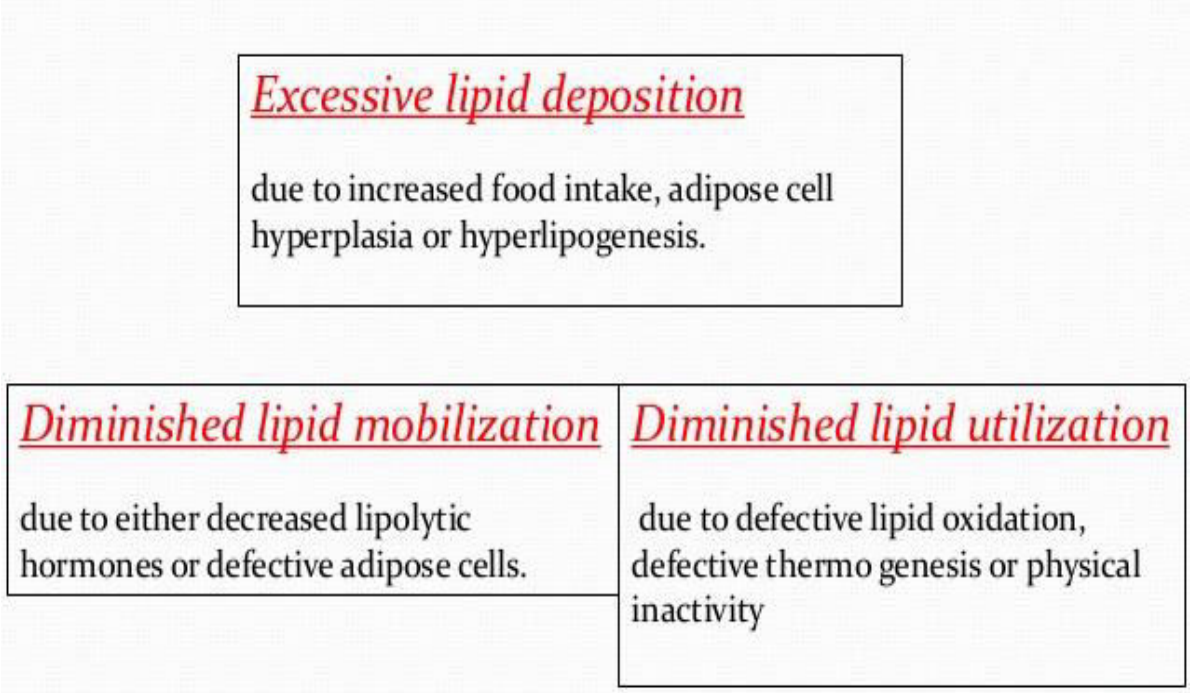
- Prader Willi Syndrome
- Bardet Moon Biedel Syndrome
- Carpenter Syndrome
- Cohen Syndrome
- Pseudohypoparathyroidism (Albright hereditary osteodystrophy)

**PATHOGENESIS OF OBESITY :**

Thus obesity results from an increased energy intake, decreased energy expenditure or a combination of both.

In its simplest terms, obesity can be considered to result from an imbalance between the amount of energy consumed in the diet and the amount of energy expended through exercise and bodily functions.

**Figure 2.Pathogenesis of Obesity:**



## PATHOLOGICAL CONSEQUENCES OF OBESITY:

Obesity has major adverse effects on health and is associated with an increased mortality, with a 50 – 100% increased risk of death from all causes compared to normal weight individuals[60].

Obesity and overweight are the second leading causes of preventable death in the developing countries accounting for about 3, 00,000 deaths per year[61].

Not only the extent of excessive body fat mass, but also their anatomic location is a risk for metabolic and cardiovascular complications[62]. Mortality rates rise and the life expectancy is shortened as obesity increases, particularly when obesity is associated with intra abdominal fat[63].

In the prospective Nurses' Health study and in the Health Professionals' study, subjects in the upper normal range BMI of 23.0- 24.9kg/m² and subjects with a BMI of $\geq$ 25kg/m² had a four to five fold increase in developing the metabolic complications of obesity.

And subjects with a BMI range of 29.0 – 30.9 kg/m² had a 27.6 fold higher risk of developing the complications[64].

It is also important to note that the duration of obesity has a strong impact on the risk of developing its complications.

An increased risk of multiple health problems associated with obesity include:

- Insulin resistance (i.e) resistance to the effects of insulin on glucose uptake, metabolism and storage, characterized by a failure of target organs to respond normally to insulin. Insulin resistance includes a central component (incomplete suppression of hepatic glucose output) and a peripheral component (impaired insulin-mediated glucose uptake in skeletal muscle and adipose tissue)
- Dislipidemia and Type 2 DM.
- Hypertension, Cardiovascular diseases (such as coronary disease, stroke and congestive heart failure)
- Pulmonary diseases and abnormalities (such as reduced chest wall compliance, increased work of breathing, increased minute ventilation, decreased functional residual capacity and expiratory reserve volume, obstructive sleep apnoea and obesity hypoventilation syndrome)
- Degenerative joint diseases, osteoarthritis
- Cutaneous diseases (such as acanthosis nigricans, fungal and yeast infections),
- Reproductive disorders (such as male hypogonadism, menstrual abnormalities and PCOS).
- Malignancies like the cancers of the esophagus, colon, rectum, pancreas, liver and prostate in males and in the females is associated with the cancers of gall bladder, bile ducts, breasts, endometrium, cervix and ovaries [65].

**Figure 3.Complications of obesity:**

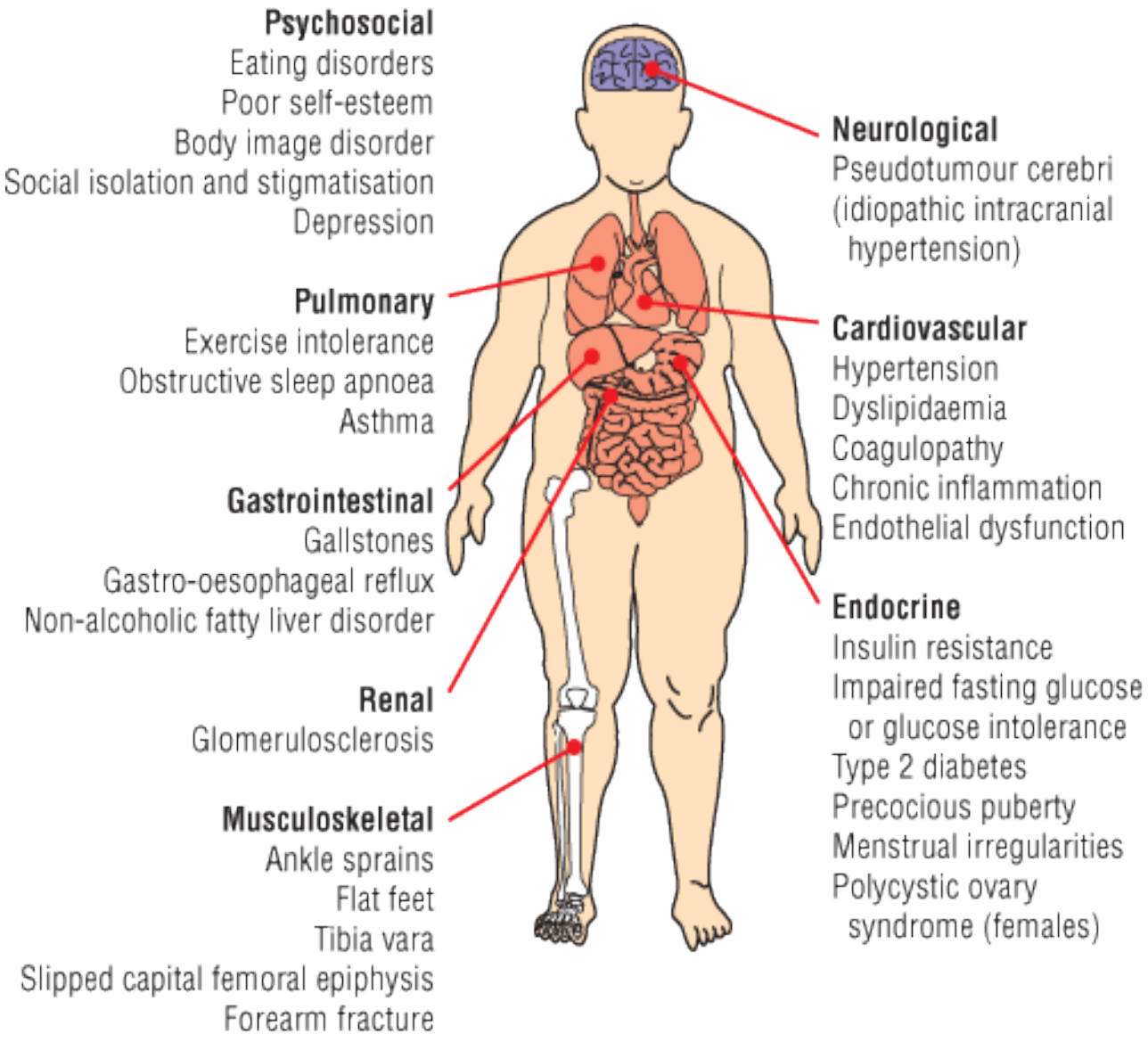
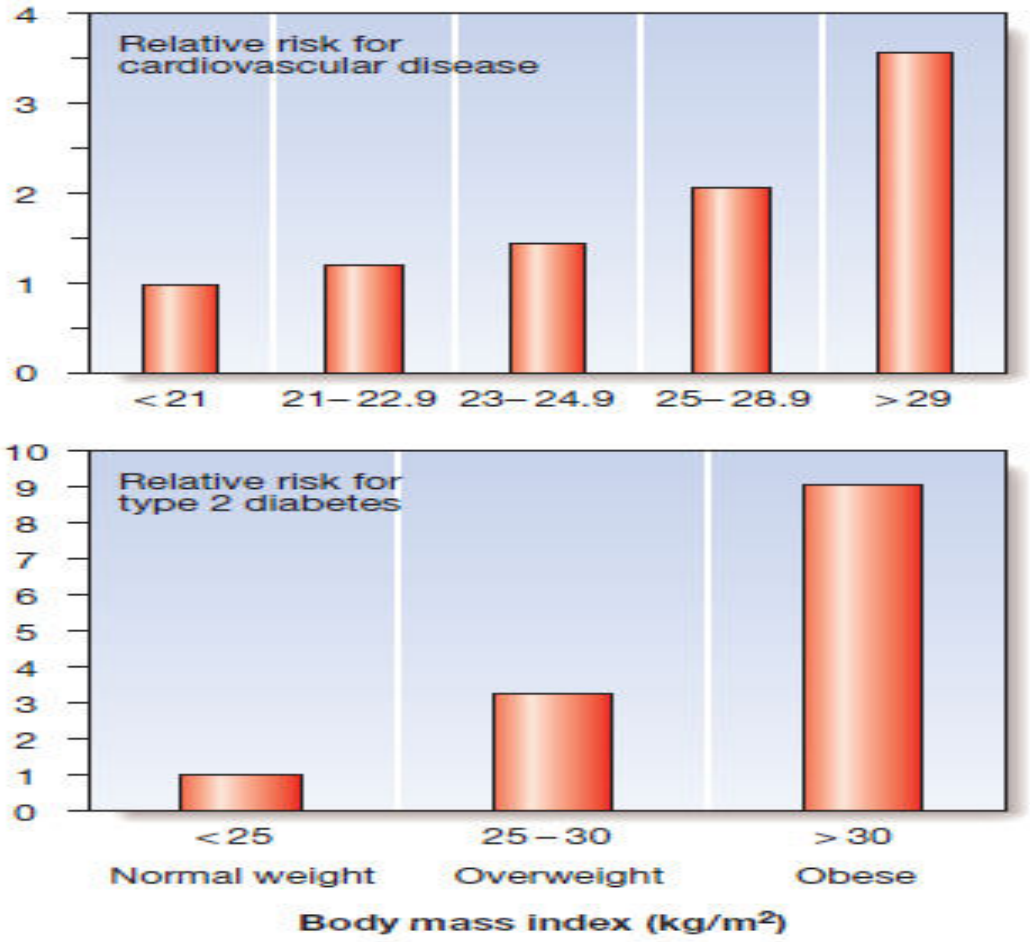


**Figure 4.Risks of Cardiovascular disease and diabetes in overweight and obese individuals:**




**Highlighting on the effects of insulin resistance:**

Hyperinsulinemia and insulin resistance are ubiquitous features of obesity increasing with weight gain. Insulin resistance is more strongly linked to intra abdominal fat.

The molecular link between visceral obesity and insulin resistance in tissues such as fat, muscle and liver has been sought after for many years[66].

The major factors include:

- Insulin itself by inducing receptor down regulation
- Free fatty acids, known to be increased and capable of impairing insulin action
- Intracellular lipid accumulation
- Various circulating peptides produced by adipocytes including cytokines and adipokines[67].

## MANAGEMENT OF OBESITY:

The primary goal of treatment is to improve obesity related co morbid conditions and reduce the risk of developing future co morbidities.

The therapy for obesity always begins with lifestyle management and may include pharmacotherapy or surgery depending on the BMI risk[68].

The success rates of maintenance of a long term weight loss, with lifestyle changes are low, ranging from 2 to 20%. The National Institutes of Health recommends a weight loss goal of 5% to 10% of the person's current weight over six months[69].

## LIFESTYLE MANAGEMENT:

Obesity care involves attention to three essential elements of lifestyle:

- Dietary habits
- Physical activity
- Behaviour modification[70]

## DIET THERAPY:

The primary focus of diet therapy is to reduce overall calorie consumption. The NHLBI guidelines recommend initiating treatment with a calorie deficit of 500 – 1000kcal/day compared to the patient's habitual diet. This calorie deficit can be accomplished by implementing substitutions or alternatives to the diet. Examples include choosing smaller portions of food, eating more fruits and vegetables, consuming more whole grain cereals,

selecting leaner cuts of meat and skimmed dairy products, reducing fried foods and other added fats and oils and drinking water instead of caloric beverages.

It is important that dietary counselling remains patient centered and that the goals are practical, realistic and achievable[71].

## PHYSICAL ACTIVITY THERAPY:

Although exercise alone is only moderate effective for weight loss, the combination of dietary modification and exercise is the most effective behavioural approach for the treatment of obesity. The most important role of exercise appears to be in the maintenance of weight loss.

Currently the minimum public health recommendation for physical activity is 30 minutes of moderate intensity physical activity on most and preferably all, days of the week. Focusing on simple ways to add physical activity into the normal daily routine through leisure activities, travel and domestic work should be suggested.

Examples include walking, using the stairs, doing home and yard work and engaging in sport activities[72].

The dietary guidelines for Americans 2005 summarises compelling evidence that at least 60 - 90 minutes of daily moderate intensity physical activity is needed to sustain weight loss[73].

**BEHAVIOURAL THERAPY:**

Cognitive behavioural therapy is used to help change and reinforce new dietary and physical activity behaviours.

Strategies include self monitoring techniques, stress management, stimulus control, social support, problem solving and cognitive restructuring to help patients develop more positive and realistic thoughts about themselves [74].

**PHARMACOTHERAPY:**

Adjuvant pharmacological treatments should be considered for patients with a BMI > 30kg/m² who also have concomitant obesity related diseases and for whom dietary and physical activity therapy has not been successful.

There are several potentials targets of pharmacologic therapy for obesity. The most thoroughly explored treatment is suppression of appetite via centrally active medications that alter monoamine neurotransmitters.

A second strategy is to reduce the absorption of selective macronutrients from the gastrointestinal tract, such as fat. These two mechanisms form the basis for all currently prescribed anti obesity agents[75].

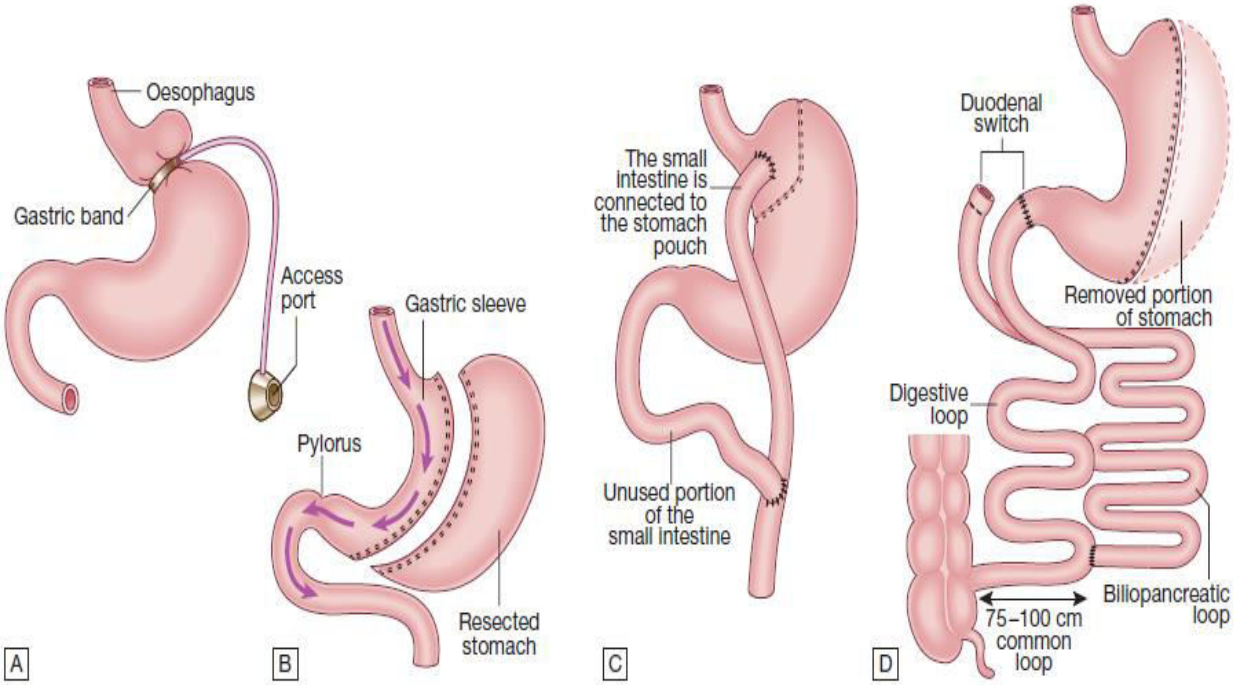
**SURGERY:**

Bariatric surgery can be considered for patients with severe obesity (BMI ≥ 40 kg/m²) and those with moderate obesity (BMI ≥ 35kg/m²) associated with a serious medical condition. Surgical weight loss functions by reducing calorie intake and depending on the procedure, macronutrient absorption[76]

**Figure 5.Surgeries for obesity:**

| Procedure | Expected weight loss (% excess weight) |
|---|---|
| Gastric banding | 50–60% |
| Sleeve gastrectomy | 50–60% |
| Roux-en-Y gastric bypass | 70–80% |
| Duodenal switch | Up to 100% |

**Figure 6. Bariatric Surgical procedures:**



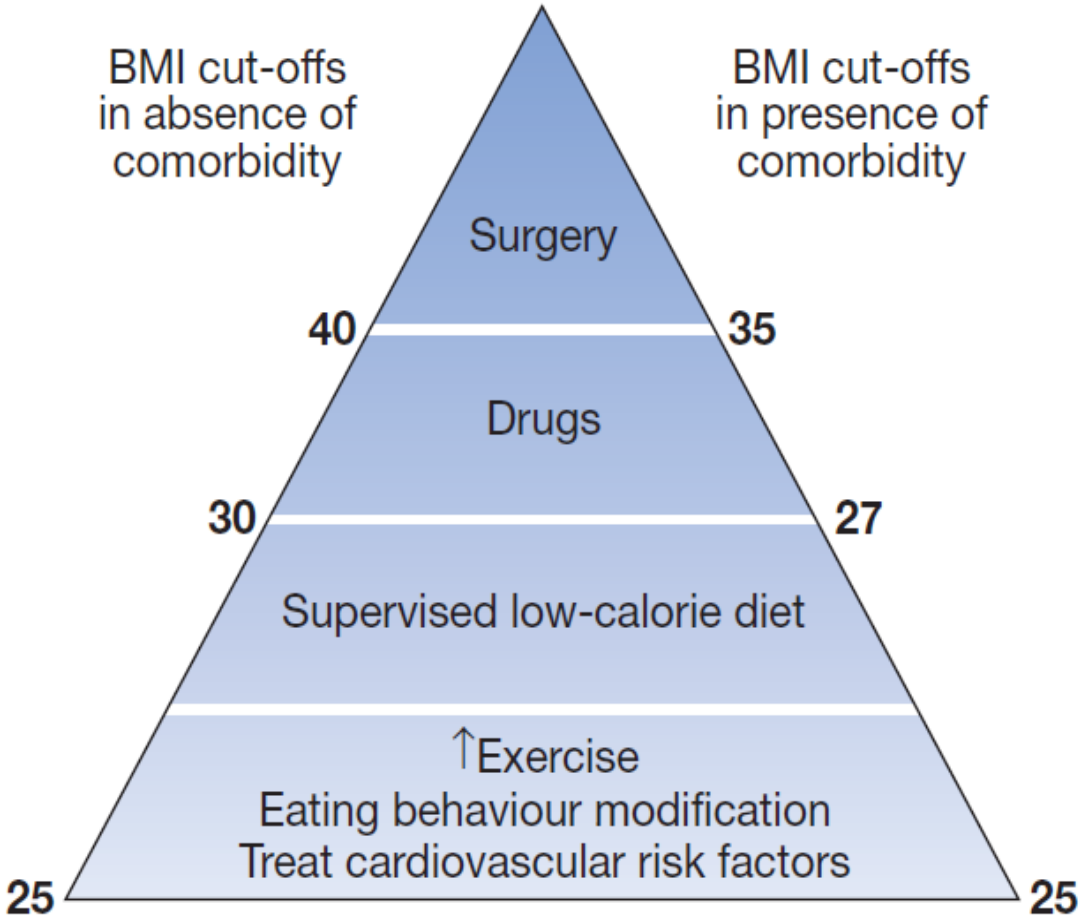

**A** – Laproscopic Banding with the option of a reservoir band and subcutaneous acess to restrict the stomach further after compensatory expansion has occurred.

**B –** Sleeve Gastrectomy.

**C –** Roux – en – Y gastric by pass.

**D –** Biliopancreatic diversions with duodenal switch.

**Figure 7.Theraupeutic options for obesity:**



# REVIEW OF LITERATURE

## REVIEW OF LITERATURE

### MEASUREMENT OF OBESITY:

There are various ways to measure the different aspects of obesity. They include Body Mass Index (BMI), skin fold thickness, waist circumference, waist to hip ratio and bio-impedance.

**Table 1 : Methods of measuring body fat & fat distribution :**

| Methods | Accuracy | Practicality | Sensitivity to change | Cheapness | Fat distribution detection |
|---|---|---|---|---|---|
| *Laboratory: 'standard' methods* | | | | | |
| Underwater weighing | ++++ | ++ | +++ | +++ | − |
| Potassium-40 counting | +++ | +++ | + | +++ | − |
| Dual-energy X-ray absorptiometry | +++ | ++ | ++ | ++ | ++ |
| Computerized tomography | +++++ | +++ | +++ | + | +++++ |
| Magnetic resonance imaging | +++++ | ++++ | +++ | + | +++++ |
| Multi-compartment models | +++ | + | + | + | − |
| Air displacement (BOD POD) | ? | ++++ | ? | ++ | − |
| *Field: anthropometric methods* | | | | | |
| Skinfold thickness | +++ | ++++ | +++ | +++++ | − |
| Circumference | +++ | ++++ | +++ | +++++ | +++++ |
| Body mass index | +++ | ++++ | +++++ | +++++ | − |

BMI has traditionally been the chosen indicator to measure body size and composition, and to diagnose underweight and overweight individuals and is one of the most basic and the most common methods.

But it is important to note that it is not a direct measure of body fat mass or distribution, and BMI measures may be skewed by very high muscle mass[51].

As proposed by **Trishnee Bhurosy and Rajesh Jeewon et al., ( 2013),** the BMI is a surrogate measure of body fatness because it is a measure of excess weight rather than excess body fat. This is one of the clinical limitations of BMI which has to be considered.

Although not a direct measure of adiposity, the most widely used method to gauge obesity is the body mass index (BMI), which is equal to weight/ height² (in kg/m²).

**Table 2 : WHO CLASSIFICATION OF OBESITY IN THE ASIAN POPULATION**

:

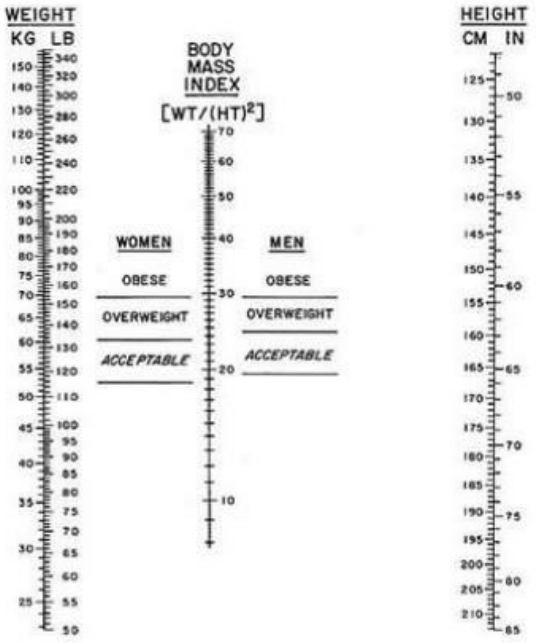
| Classification | BMI ($kg/m^2$) | Risk of co-morbidities |
|---|---|---|
| Underweight | < 18.5 | Low (but increased risk of other clinical problems) |
| Normal range | 18.5 – 22.9 | Average |
| Overweight | ≥ 23 | |
| At risk | 23 – 24.9 | Increased |
| Obese I | 25 – 29.9 | Moderate |
| Obese II | ≥ 30 | Severe |

A BMI between 25 and 30 should be viewed as medically significant and worthy of therapeutic intervention, especially in the presence of risk factors that are influenced by adiposity, such as hypertension and glucose intolerance[52].

**Figure 8:NORMOGRAM FOR DETERMINING THE BODY MASS INDEX :**

An alternative to calculating the BMI is a **nomogram**. Simply use a ruler to draw a line from the body mass (weight) to the height of a person. Where it intersects the W/H² line the person's BMI can be determined. Now use the table to assess their BMI status.

| BMI | Status |
|---|---|
| Below 18.5 | Underweight |
| 18.5 – 24.9 | Normal |
| 25.0 – 29.9 | Overweight |
| 30.0 and Above | Obese |




However, alternative measures that reflect abdominal adiposity, such as waist circumference and the waist–hip ratio have been suggested as being superior to BMI in predicting CVD risk as proposed by **Huxley et al., 2010**[50]. These are also called the field methods to determine central obesity.

The circumference of the waist is widely used as a simple measure of body fatness [53].

Adult waist circumference cut points are:

- Increased risk of health problems: Men ≥ 94cm & Women ≥ 80cm
- Greatly increased risk of health problems: Men ≥ 102cm & Women ≥ 88cm

The waist hip ratio of > 0.9 in women and > 1.0 in men is said to be abnormal.

These cut off values are as proposed by the **WHO expert report in December 2008**.

**Table 3: Sex specific waist circumference measurements for identification of individuals at increased health risk to intra abdominal fat accumulation :**

| Gender | Risk (Warning border) (=BMI>25) | High risk (Action border) ( =BMI>30) |
|---|---|---|
| Male | ≥94 | ≥102 |
| Female | ≥80 | ≥88 |

**Table 4: Action levels to identify overweight and obese men & women with increased abdominal fat:**

| Waist circumference | | Approximate equivalents | | | |
|---|---|---|---|---|---|
| Action level | cm | Body mass index | Waist-to-hip ratio | Classification of health risks | Weight management |
| Men | | | | | |
| Action level 1 | ≥ 94 | ≥ 25 | ≥ 0.95 | Increased health risks | Prevent further weight gain, try to get down to below action level 1 (94 cm) |
| Action level 2 | ≥ 102 | ≥ 30 | ≥ 0.95 | High health risks | Seek advice to lose weight, aim for 5–10% weight loss permanently |
| Women | | | | | |
| Action level 1 | ≥ 80 | ≥ 25 | ≥ 0.80 | Increased health risks | Prevent further weight gain, try to get down to below action level 1 (80 cm) |
| Action level 2 | ≥ 88 | ≥ 30 | ≥ 0.80 | High health risks | Seek advice to lose weight, aim for 5–10% weight loss permanently |

An excess abdominal fat assessed by measurement of the waist circumference and waist hip ratio, is independently associated with higher risk for insulin resistance, diabetes mellitus and cardiovascular diseases and is a surrogate for visceral adipose tissue as documented by **Yusuf et al., (2004) in the Interheart study**[54].

A careful analysis of the relationship between obesity and adult- onset diabetes confirms that abdominal obesity is an important risk factor, even after controlling for age, smoking and family history,as the waist circumference correlates more closely with abdominal adipose. tissue[55].

Waist to hip ratio examines fat distribution and there are established links between waist circumference alone and health risk[56].

This is based largely on the rationale that increased visceral adipose tissue is associated with a range of metabolic abnormalities, including decreased glucose tolerance, reduced insulin sensitivity and adverse lipid profiles, which are risk factors for type 2 diabetes and CVD as documented by **Huxley et al., 2010**[57].

Also **Kiessebah et al., and Krotkiewski et al., in the 1980s** demonstrated that hypertension, hypertriglyceridemia, hyperinsulinemia and glucose intolerance were increased in subjects with a high waist hip ratio.

**Gothenberg et al**., in the year 2010 put forward that the waist hip ratio was a predictor of the future development of diabetes, myocardial infarction, angina pectoris, stroke and death independent of BMI[58].

Thus from the reviewed literature it has been concluded that:

• "Waist circumference and waist–hip ratio are both related to an increased risk of all- cause mortality, throughout the range of adult BMIs".

• "Waist circumference and waist–hip ratio are strongly predictive in young and middle aged adults".

• "Waist circumference alone could replace waist–hip ratio and BMI as a singrisk factor for all- cause mortality".

Other most accurate and sophisticated approaches to quantify an abdominal obesity include dual-energy X-ray absorptiometry - DEXA (under water weighing), CT or MRI and electrical impedance. Out of all these methods, CT and MRI scans are the imaging techniques to provide a direct assessment of the intra abdominal visceral adipose tissue and these have made it possible to precisely measure the specific adipose tissue depots[59.]

**Figure 9.Measurement of Hip Circumference:**

**Figure 10.Measuring waist circumference:**

**Figure 11.Measurement of skin fold thickness using skinfold callipers:**

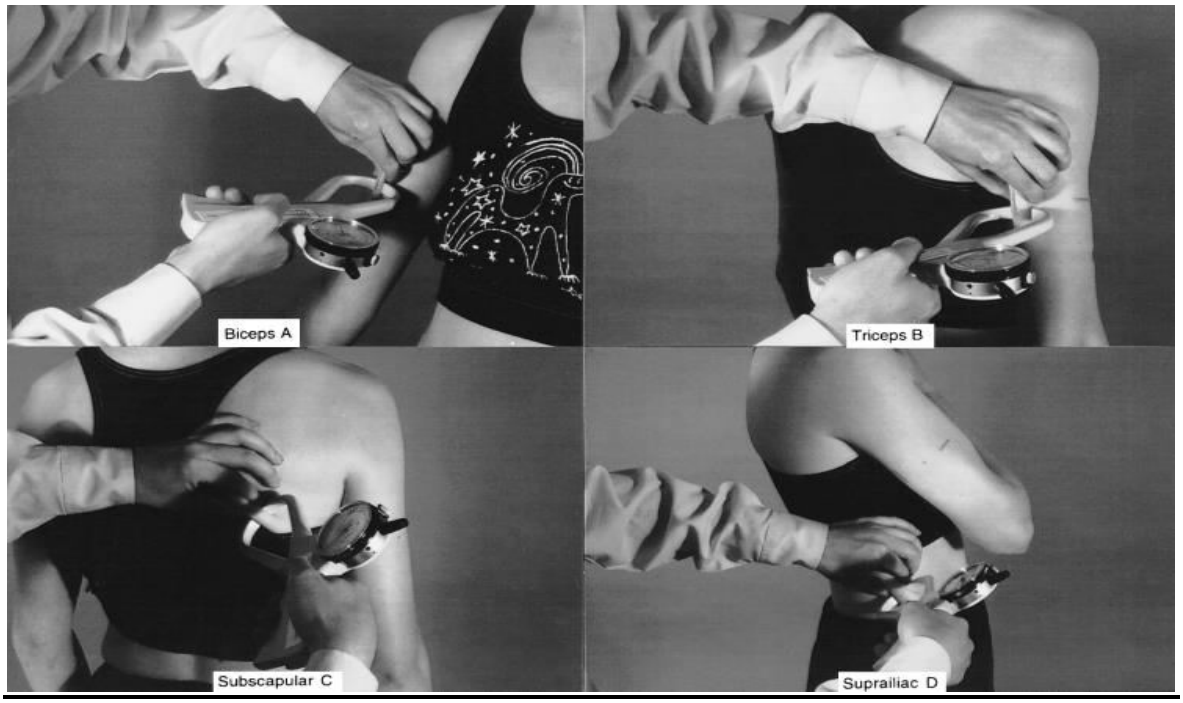
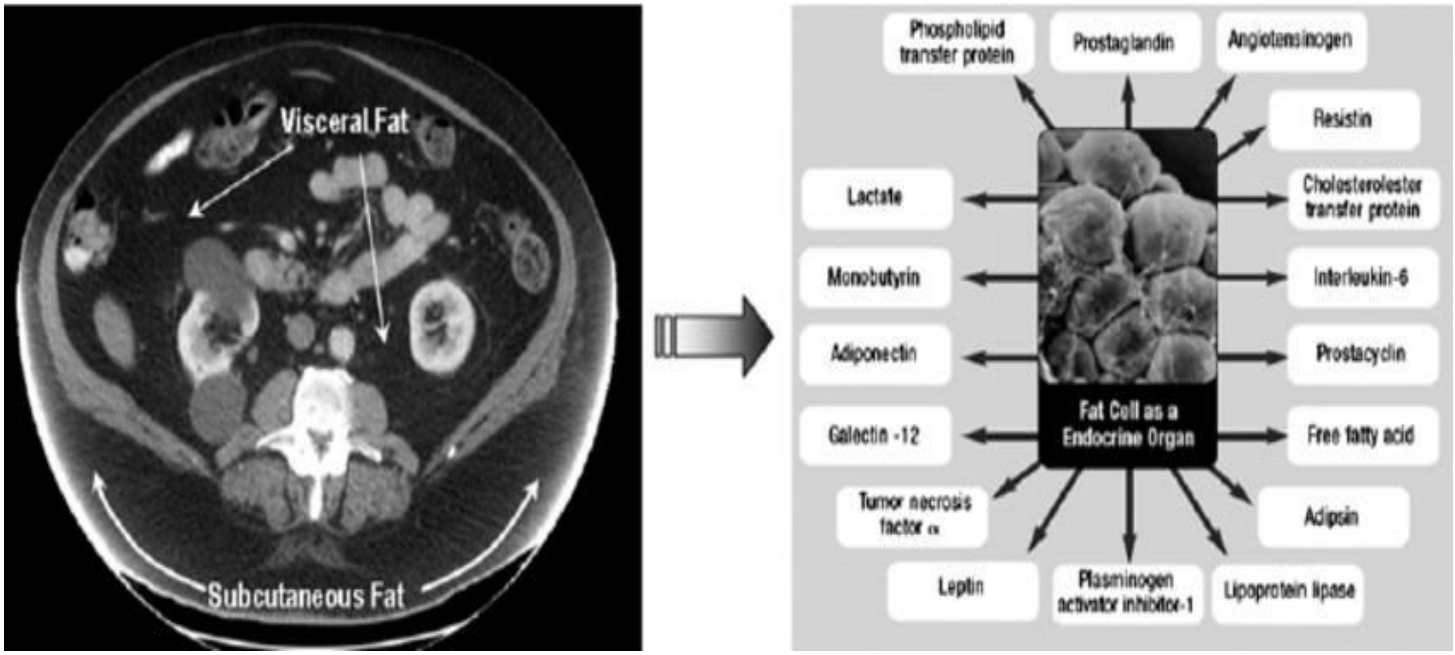



**Figure 12.Measuring total body fat by underwater weighing:**

**Figure 13.Magnetic resonance imaging scanner to image**

**Figure 14.Magnetic resonance imaging showing the visceral and subcutaneous fat depots:**



## OBESITY AND INSULIN RESISTANCE:

## ADIPOSE TISSUE:

Adipose tissue is a highly active metabolic and endocrine organ, the adipose organ that significantly contributes to the regulation of body's homeostasis as presented by **Siiteri et al., 1987 and Mohamed Ali et al., 199877**. Adipose tissue or fatty tissue is an anatomical term for loose connective tissue composed of fat cells or adipose cells called adipocytes. The adipose organ is a multi depot organ with a complex shape and its main role is to store energy in the form of lipids, although it also cushions and insulates the body. It also functions as a reserve of nutrients.

The adipose tissue comprises of adipocytes, which comprise the highest percentage of cells within adipose tissue. There are also other cell types present which are collectively termed as stromal vascular fraction (SVF) of cells.

SVF includes preadipocytes, fibroblasts, adipose tissue macrophages, and vascular endothelial cells[78].

Adipose tissue is derived from preadipocytes and is composed of the lipid storing adipose cell and a stromal/ vascular compartment in which cells including preadipocytes and macrophages reside.

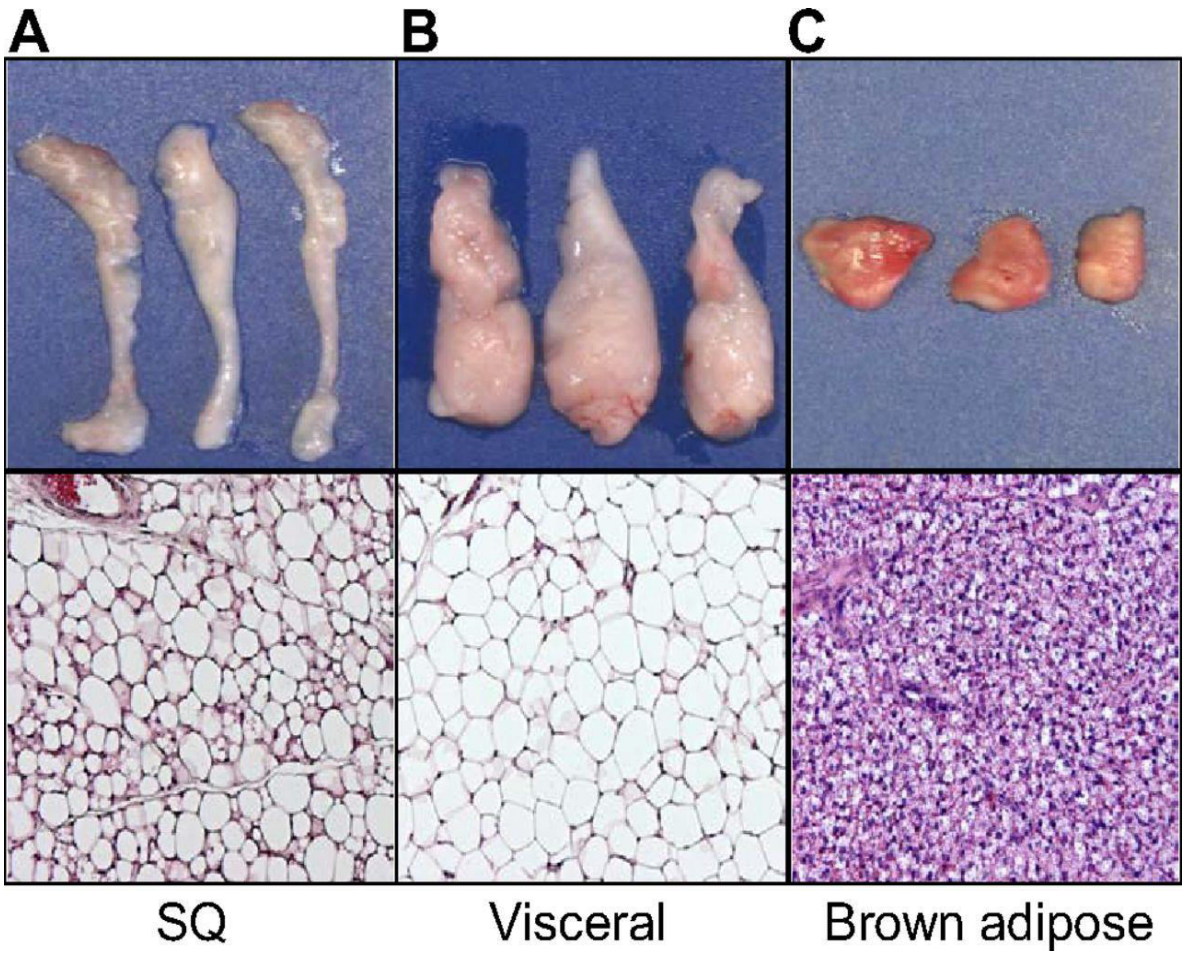
The process by which adipose cells are derived from a mesenchymal preadipocyte is through an orchestrated series of differentiation steps mediated by a cascade of specific transcription factors.

In humans there are subcutaneous fat (adipose tissue is located beneath the skin and is continuous with the dermal adipose tissue) and visceral depots or the visceral fat located around the internal organs[79].

The key difference between these depots of fat may lie in their vascular anatomy, with the intra-abdominal fat draining into the portal vein and thence directly to the liver. Thus the adipokines that are released from adipose tissue may be at higher concentration in the liver and hence induce insulin resistance and promote

Type 2 diabetes mellitus.

**Figure 15. Types of adipose tissue:**



Adipose tissue is found in specific locations, which are referred to as adipose depots. There are several adipose tissue depots. - (i.e) inside the thorax (mediastinic) and the intra abdominal fat depots.

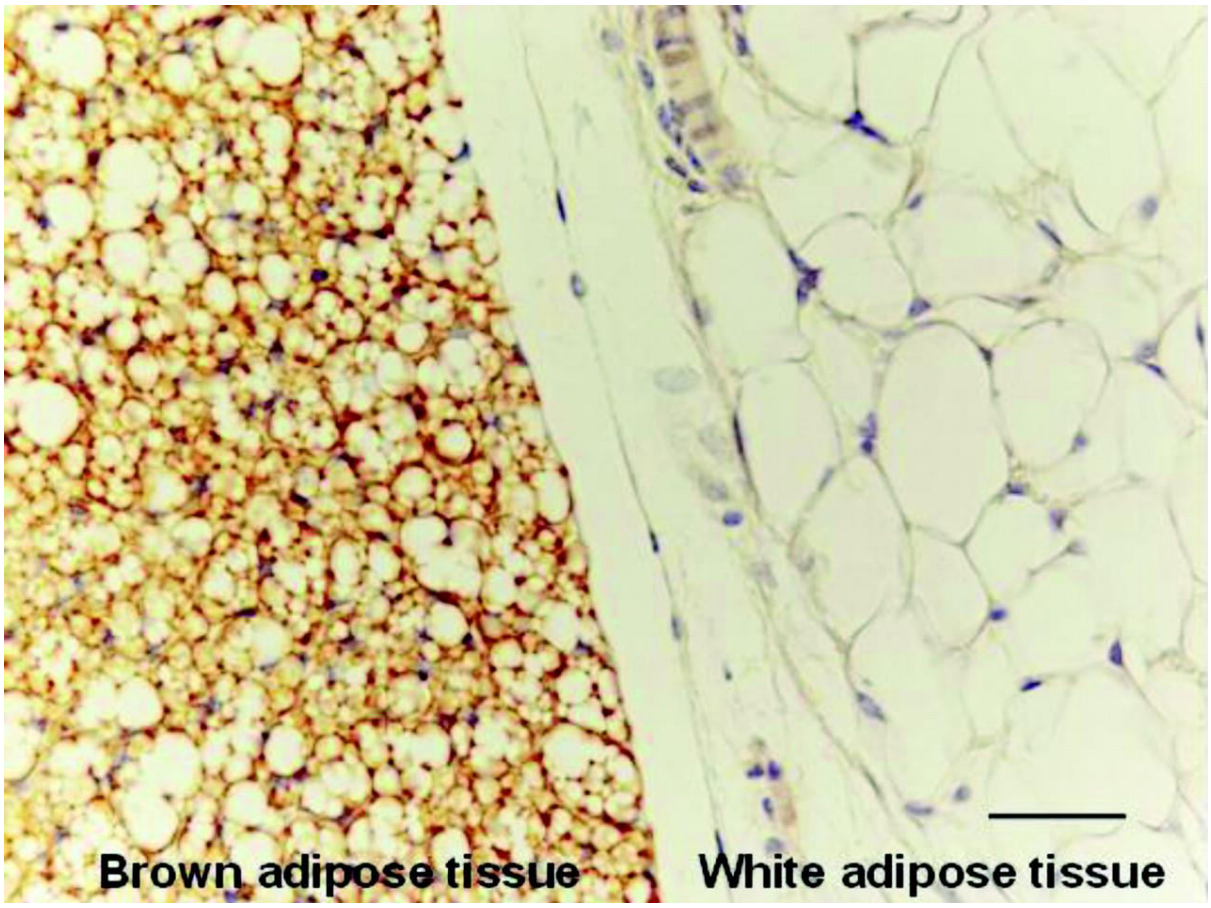
**Table 5: MAJOR ABDOMINAL DEPOTS IN HUMANS:**

| DEPOT | APPROXIMATE SIZE ( kg) | COMMENTS |
|---|---|---|
| 1.**Subcutaneous** : ( anterior + posterior) | 1-20 | The most variable of the abdominal depots |
| 2. **Intra abdominal** : | | |
| 2 a. Omental | 0.5 - 3 | Visceral depots; drain mostly to portal vein |
| 2 b. Mesenteric | 0.5 - 2 | Visceral depots; drain mostly to portal vein |
| 2 c. Perirenal | 0.5 - 2 | Retroperitoneal |

Of the intra abdominal depots, the most important attention and focus is to be given to the omental and mesenteric depots (visceral fat depots) [80].

The colors of the organ are white and brown. The white adipose tissue is made mainly by white adipocytes. The brown adipose tissue is made mainly by brown adipocytes. Both white and brown adipose tissues are organised into a real organ, with a complex multi-depot organisation.

**Figure 16.White and Brown Adipose tissue :**




White adipose, provides insulation and serves as an energy store during starvation or great exertion, and forms pads between organs[81]. When muscles and other tissues need energy, certain hormones bind to the adipose cells and trigger the hydrolysis of triacylglycerol, resulting in the release of energy-rich fatty acids and glycerol—a process known as lipolysis.

The enzyme that is responsible for hydrolysis is lipase, which is present in the blood, certain gastrointestinal juices, and adipose tissue. Lipase is activated by the hormones epinephrine, norepinephrine, glucagon, and adrenocorticotropin, which bind to adipocytes.

White adipose tissue also is a source of a number of different hormones, which serve various roles in metabolism and endocrine function[82].

Brown adipose, found mainly in newborn animals, generates heat and actually consumes energy.

In humans, the percentage of brown adipose decreases with age.

In the animals that hibernate such as the grizzly and black bears, brown adipose tissue plays an important role in their survival. Species that hibernate, experience a drop in their body temperature and slowing of metabolism during winter dormancy, which allows them to conserve energy.

By consuming energy,the brown adipose tissue releases heat, which is vital for awakening and emergence from dormancy[83]. Brown adipose tissue is red in colour. Its colour and heat-generating properties are imparted by the abundance of organelles known as mitochondria found in brown fat cells. Mitochondria are the energy-producing components of cells[84].

The weight of the human adipose organ of lean adults is about 8 to 18% of body weight in males and 14 to 28% in females[85].

Development of the human adipose organ extends for a long period, until puberty, mainly through proliferation and there is an increase in size mainly during the first year.

The adipocyte size is correlated to the amount and the percentage of fat mass. In massively obese humans, the adipose organ can increase four times and reach 60 to 70% of body weight[86].

The adipose organ reduces its volume and size, when there is a negative energy balance. This reduction in size of adipocytes is important because the size of adipocytes correlates with insulin sensitivity[87].

Adipose mass increases by enlargement of adipose cells through lipid deposition, and also by an increase in the number of adipocytes. "It has been proposed that the white adipose tissue of humans is infiltrated with the macrophages and this level of infiltration correlates with body mass index and the mean size of the adipocytes"[88].

Obese adipose tissue is also characterised by increased numbers of infiltrating macrophages and this infiltration seems to be an important cause for the insulin resistance associated with obesity[89].

The distribution of adipose tissue in the different anatomic depots also has substantial implications for morbidity. Specifically, the intra abdominal fat has more significance than the subcutaneous fat and is strongly linked to the most important complications of obesity, such as insulin resistance, diabetes, hypertension, hyperlipidemia and hyperandrogenism in women. This was as proposed by **Brian et al.,(2007)**[90]**.**

The mechanism underlying this association is that intra abdominal adipocytes are more lipolytically active than those from other depots. Release of free fatty acids into the portal circulation has adverse metabolic actions, especially on the liver.

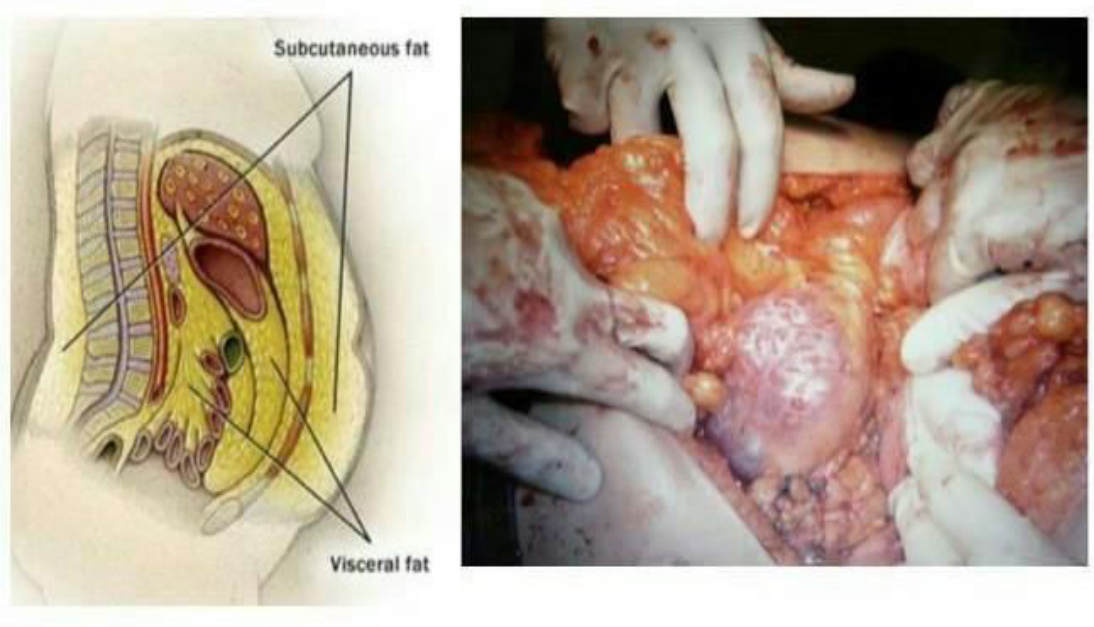
## VISCERAL ADIPOSE TISSUE AND INSULIN RESISTANCE :

**Sushter et al., in the year 2012** submitted that Asian populations have greater visceral adipose tissue or percentage of body fat at any given waist circumference[91].

Visceral fat is body fat that is stored within the abdominal cavity and is therefore stored around a number of important internal organs such as liver, pancreas and intestines.

"Visceral fat is also known as 'active fat' as it plays a unique and potentially dangerous role in affecting how our hormones function".

**Figure 17.Visceral Fat :**




Carrying a high amount of visceral fat is known to be associated with insulin resistance, which can lead to an increased risk of many health conditions such as

type 2 diabetes mellitus, heart diseases, breast cancer, colorectal cancer and Alzheimer's disease. Also, the hyperinsulinemia that accompanies insulin resistance would magnify and mediate the detrimental effects of visceral obesity[92].

Research studies at the Harvard University in the year 2012 have noted that around 10% of our total fat is likely to be stored as visceral fat. Therefore if you are carrying higher amounts of body fat than is recommended, it is therefore more likely that you are also storing more visceral fat than that is healthy[93].

Although much of the details of the mechanisms by which an increased adipose tissue mass causes a systemic insulin resistance remains unknown, the past several years have witnessed an explosive increase in our understanding of what may now be referred to as the adipo-insulin axis.

"There are related possibilities that insulin resistance and hyperinsulinemia, in addition to being caused by obesity, can also contribute to the development of obesity"[94].

## INSULIN ACTION IN ADIPOCYTE:

Adipocytes are one of the most highly insulin-responsive cell types and insulin is a critical regulator of all aspects of adipocyte biology. Insulin promotes adipocyte triglyceride stores by a number of mechanisms, including fostering the differentiation of preadipocytes to adipocytes, by stimulating glucose transport, triglyceride synthesis (lipogenesis) and by inhibiting lipolysis .

By stimulating lipoprotein lipase activity in adipose tissue, insulin also increases the uptake of fatty acids derived from circulating lipoproteins. The metabolic effects of insulin are mediated by a wide range of tissue-specific actions that involve rapid changes in protein phosphorylation and function, as well as changes in gene expression[95].

The activation of the insulin receptor tyrosine kinase, is the initial signal for the action of insulin and this would result in the phosphorylation of insulin receptor substrates on multiple tyrosine residues.

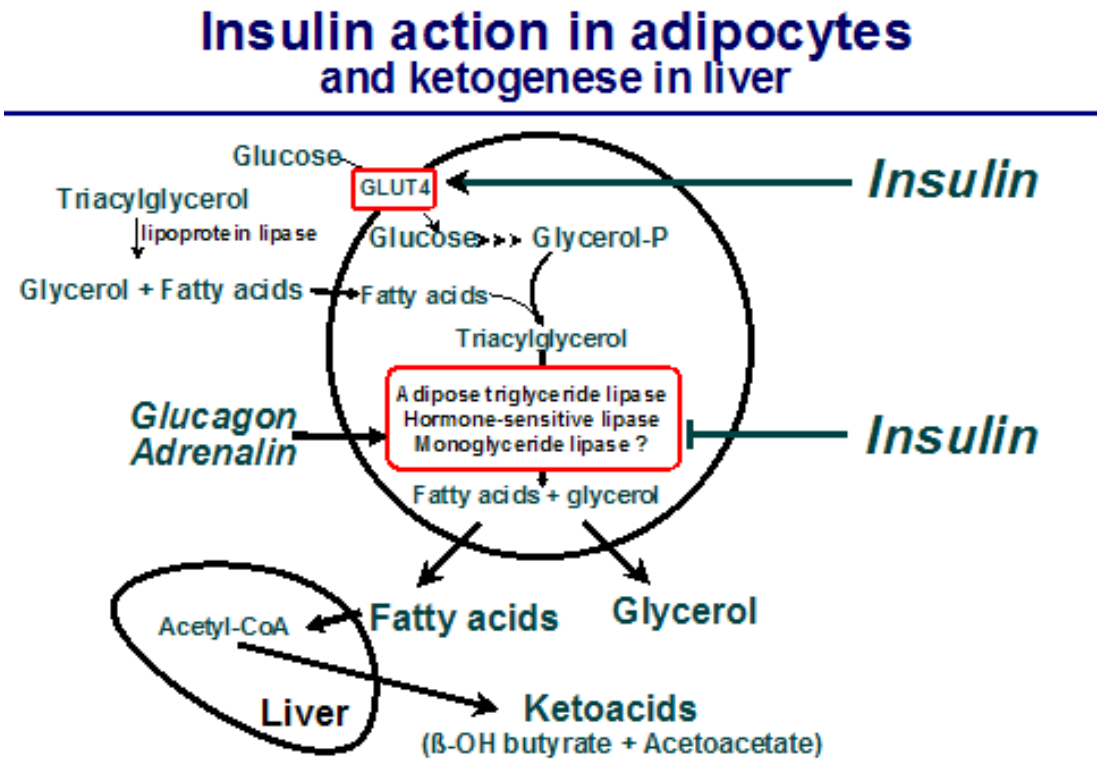
These phospho tyrosine residues act as docking sites for many SH2 domain–containing proteins, including the p85 regulatory subunit of phosphoinositide 3′ kinase (PI3K). Binding of the p110 catalytic subunit of PI3K to p85 activates the lipid kinase that promotes glucose transport[96].

Insulin also activates the ras–mitogen-activated protein kinase (ras-MAPK) signaling cascade and this pathway appears to be important for the mitogenic effects of insulin. The action of insulin in the adipocytes, also involves changes in gene transcription.

The transcription factor ADD-1/SREBP-1c (adipocyte determination and differentiation factor-1/sterol regulatory element–binding protein-1c) may play a critical role in the actions of insulin to regulate adipocyte gene expression , by inducing genes involved in lipogenesis and repressing those involved in fatty acid oxidation.[97]

Thus the overall action of insulin on the adipose tissue is to stimulate fat storage and inhibit mobilisation.

**Figure 18. Action of Insulin on Adipose tissue :**



## OBESITY AND INSULIN RESISTANCE:

Insulin resistance in obesity is manifested by an impaired suppression of hepatic glucose output and a decreased insulin-stimulated glucose transport and metabolism in the adipocytes and skeletal muscle.

These functional defects are caused due to the impaired insulin signaling in all three target tissues and in the adipocytes. It would also result from the downregulation of GLUT4, which is the major insulin-responsive glucose transporter.

In both the muscle and adipocytes, the binding of insulin to its receptor, the receptor phosphorylation and tyrosine kinase activity, and phosphorylation of IRSs are all reduced.

There are also tissue-specific alterations: In the adipocytes of the obese humans, IRS-1 expression is reduced, resulting in a decreased IRS-1–associated PI3K activity, and IRS-2 becomes the main docking protein for PI3K. Whereas in the skeletal muscles of the obese individuals, IRS-1 and IRS-2 protein levels are normal but PI3K activity associated with both IRSs is impaired[98].

"The signaling defects in obesity may be due to an increased expression and activity of several protein tyrosine phosphatases (PTPs), which dephosphorylate and thus terminate the signals propagated through the tyrosyl phosphorylation events"[99].

Interestingly, insulin sensitivity is present in the muscle and liver but not in the adipocytes. Other mechanisms also contribute to insulin resistance in obesity. In morbid obesity, the expressions of the molecules that signal insulin are reduced in skeletal muscle.

In all forms of obesity, the downregulation of GLUT4 is a major factor contributing to the impaired insulin-stimulated glucose transport in adipocytes .

However, in the skeletal muscle of obese and diabetic individuals, GLUT4 expression is normal and a defective glucose transport appears to be due to an impaired translocation, docking, or fusion of GLUT4-containing vesicles with the plasma membrane[100].

Although insulin resistance is characteristic of obesity and type 2 diabetes, it is not postulated that all the actions of insulin are impaired in individuals with both these conditions. There occurs an excess of lipid storage in adipose tissue and the lipogenesis in the liver is increased, whereas other insulin effects related to glucose homeostasis are impaired.

## REDUCED GLUCOSE DISPOSAL INTO ADIPOSE TISSUE IN OBESITY:

Suppression of the glucose production from the liver and an increased uptake of glucose into the muscle and fat, both cause insulin to lower the blood glucose levels[101].

Muscle has long been considered the major site of insulin-stimulated glucose uptake in vivo, with adipose tissue contributing relatively little to total body glucose disposal. This conclusion is supported from the fact that measurements of 2-deoxyglucose uptake in vivo show at least ten times more glucose per milligram of tissue going into muscle than into white adipose tissue (WAT) [102].

Because muscle mass is considerably greater than WAT mass, at least in lean humans, this observation has been taken to indicate the prominent contribution of muscle to glucose disposal. The higher glucose transport into brown adipose tissue (BAT) and the small mass of BAT make this an unlikely site to account for large amounts of total body glucose uptake[103].

Thus, it is unlikely that a diminished glucose uptake into the fat cells could account for a diminished whole body glucose uptake in the obese individuals. Over expression of GLUT4 selectively in fat enhances the glucose tolerance and body's sensitivity to insulin.

"Knocking out *GLUT4* selectively from fat, results in the development of insulin resistance which is similar to that seen with muscle-specific knockout of *GLUT4"*[104].

Likely candidates for indirect effects are FFA, leptin, or TNF-α, all of which are known to affect glucose homeostasis[105].

Undoubtedly there are other undiscovered molecules that are secreted from the fat cells that influence the systemic metabolism.

From studies where humans were treated with the β3 adrenergic agonist CL316,243, further support for a potential direct role of adipocytes in regulating systemic glucose homeostasis has been obtained . Since β3 adrenergic receptors are expressed almost exclusively in fat, their effects would be initiated by alterations in fat[106].

Treatment with CL316, 243 results in enhanced sensitivity of both whole body glucose uptake and suppression of hepatic glucose production. These effects are accompanied by increased glucose uptake in adipose tissue. Thus, increasing glucose uptake selectively in fat with β3 adrenergic receptor agonists would improve the whole body glucose uptake, indirectly resulting in increased insulin sensitivity in liver[107].

Alternatively, β3 agonists may work by changing the release of some products from the adipocytes that influences systemic insulin sensitivity[108 &109].

## THE SIGNIFICANCE OF VISCERAL FAT FOR INSULIN RESISTANCE:

The main adverse effect of an abdominal obesity is on the action of insulin, particularly in the liver, muscle and adipose tissue[110 & 111].

The underlying cause of the adverse effects of an abdominal type of fat distribution is that intra abdominal fat cells or adipocytes exhibit a differing expression profile and are lipolytically more active and have got the highest rate of lipolysis.

This high lipolytic capacity in the visceral adipocytes is due to a greater sensitivity to stimulatory β - adrenoceptors and lower sensitivity to the antilipolytic α – adrenoceptors as well as to the receptors for other inhibitory agents such as adenosine and insulin. This was as proposed by **Mauriege et al., 1987 and Van Harmelen at al., (1997)**[112].

Thus they discharge free fatty acids, also called 'bad actors' at a high rate and interfere with the insulin sensitive glucose metabolism. They show a greater accumulation of macrophages and lymphocytes, indicating a greater proinflammatory activity.

Visceral adipose tissue also has a much higher blood vessel and nerve density leading to a much greater metabolic activity. The venous drainage from the visceral adipose tissue depots drain directly into the portal vein and thus the liver is directly exposed to the its metabolic products such as fatty acids and proteins released from this active fat depot promoting insulin resistance in the liver[113].

This would in turn increase the intraportal FFA levels and flux and an increased triglyceride storage, which might inhibit insulin clearance and promote insulin resistance. These adverse effects are called as 'lipotoxicity'[114].

Thus the inflammatory process is detected, not only at the level of adipose tissue, but also affect the liver and other organs.

As enlarged visceral fat depots are frequently associated with fat accumulation in the liver, it was hypothesised that secretory products from the visceral adipose tissue may directly cause hepatic insulin resistance. This mechanism by which insulin resistance and many of its deleterious effects arise due to the liberation of high rates of NEFA is called the Portal theory as propounded by **Arner et al., 1997**[115].

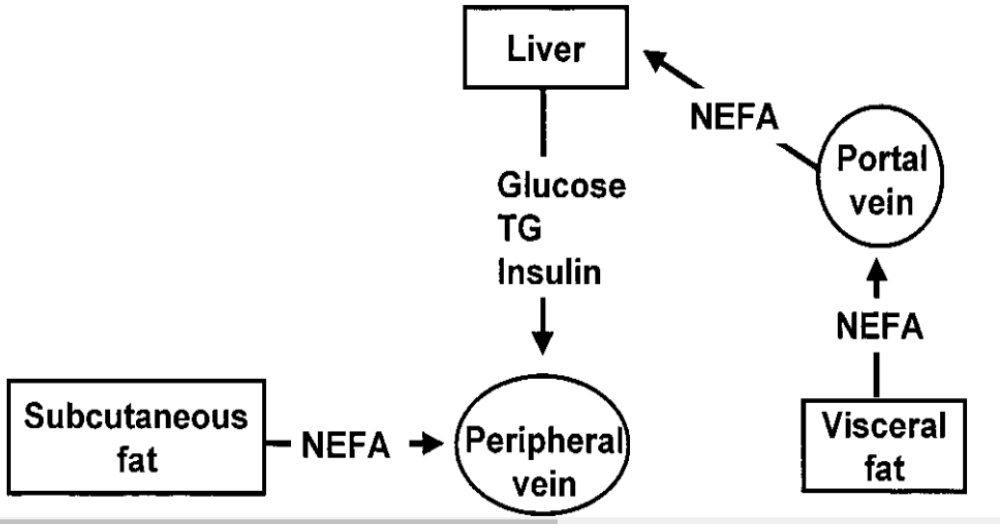
Thus the various potential mechanisms of how the visceral adiposity causes an insulin resistance are as follows:

1. The free fatty acids and the non-esterfied fatty acids (NEFA) are the damaging molecules and the important products of the visceral adipocytes ( especially of the omental and the mesenteric adipose tissue depots) that cause insulin resistance by interfering with the hepatic insulin removal[116].

Thus causing a hyperinsulinemia. This was as said by **Ostman et al., (1979)** and **Engfeldt & Arner et al., (1988)**. They also highlighted that hyperinsulinemia can cause an insulin resistance by down regulating the insulin receptors and desensitizing post receptor pathways[117].

2. The anatomical positioning of the visceral adipose tissue depot, also plays an important role in the pathogenesis of insulin resistance by causing a compression of the viscera[118 & 119].
3. The over expression of the cytokines, genes and peptide hormones which possess the capacity to influence local adipocyte biology as well as have marked effects on the systemic metabolism in the brain, liver, muscle, β cells, gonads, lymphoid organs and systemic vasculature[120].

**Figure 19.Visceral fat and Insulin resistance :**




To summarise, chronic over nutrition with a high fat, high sugar diet and as a consequence an accumulation of body fat is the primary cause of chronic inflammation in obesity and may promote the development of systemic insulin resistance which affects many tissues including the liver, muscle and brain.

**Figure20:Lipotoxicity Theory of insulin resistance:**

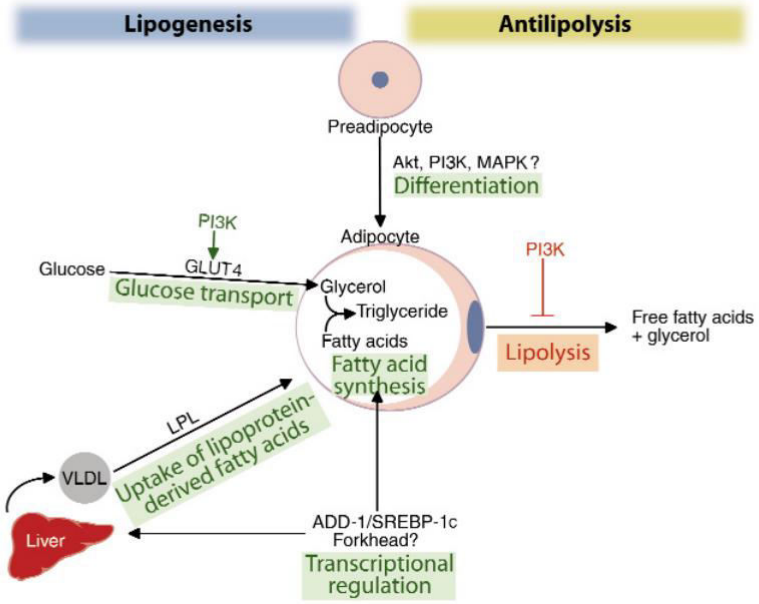
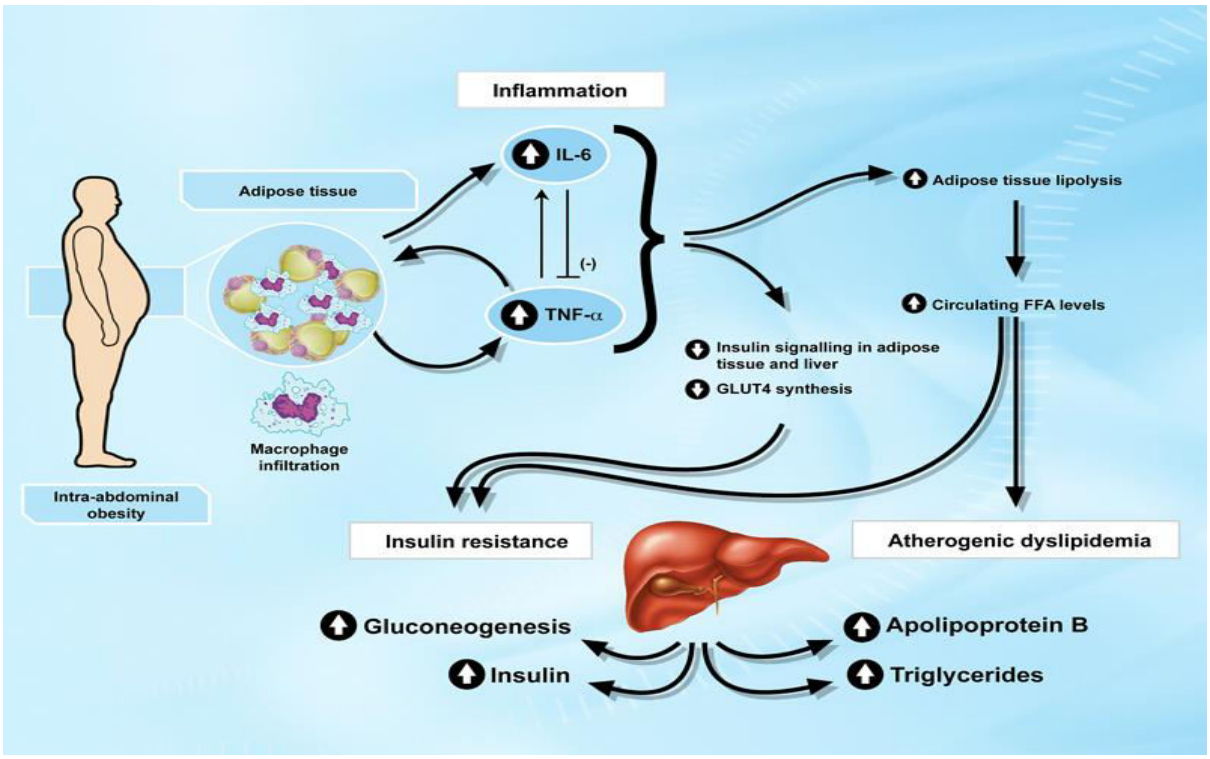



**Figure 21.Mechanism of how visceral adiposity causes Insulin resistance:**



**ADIPOKINES** :

Evidence for the association of obesity with inflammation dates back at least to the 1960s, when population studies found that obesity increases the circulating concentration of fibrinogen and other acute phase factors.

The circulating concentration of more than a dozen proinflammatory cytokines such as tumor necrosis factor (TNF), monocyte chemoattractant protein [MCP]-1, interleukin [IL]-6, acute-phase reactants (C-reactive protein [CRP]), serum amyloid A [SAA]-3, lipocalin and procoagulant proteins plasminogen activator inhibitor [PAI]-1, Factor VII are now known to be increased by obesity, as reported by **Hotamisligil et al., in the early 1990s**.

Although the adipocyte has generally been regarded as an energy storage depot for fat, it is also an endocrine cell that releases numerous molecules in a regulated fashion. These include the energy balance – regulating hormone leptin, bioactive peptides called adipokines and cytokines such as tumour necrosis factor ( TNF α ) and interleukin ( IL – 6 ), complement factors such as factor D ( adipsin ), prothrombotic agents such as plasminogen activator inhibitor I, and a component of the blood pressure regulating system, angiotensin.

**Lago et al in the year 2007** identified that these factors played a role in lipid homeostasis, insulin sensitivity, blood pressure control, coagulation and vascular health, insulin action, energy metabolism, inflammation, cell growth and are likely to contribute to obesity related pathologies[121,122].

An excess of fat accumulation would cause the over secretion of the deleterious adipokines and hypo secretion of the advantageous ones.

The visceral adipocytes secrete adipokines and cytokines which play an additional role in systemic complications of obesity. Thus adipokines have emerged to be a new link between obesity and insulin resistance.

**Table 6: Various adipokines and cytokines secreted from the visceral adipose tissue :**

Circulating Proteins Secreted by Adipose Tissue and That May Link Obesity to Insulin Resistance and Diabetes

| *Adipokine* | *Effect on insulin response* | *Change in blood concentration* | *Tissue expression* | *Expression in VF vs SCF* |
|---|---|---|---|---|
| Resistin | Impairment | Elevated in rodent models of genetic and diet-induced obesity | Mouse adipocytes *(43)*, human non-adipocyte fat cells *(51)* | Markedly higher in VF of rodents *(25)*. Equal in VF and SCF of humans *(48)* |
| TNF-α | Impairment | May be elevated in human obesity and insulin resistance | Adipose tissue macrophages *(14)*, liver and muscle *(26)* | Higher in VF of rodents *(25)* |
| IL-6 | Impairment | Dramatic elevation in obese humans *(54)* | Non-adipocyte fat cells, immune cells, skeletal muscle *(2)* | Higher in VF of humans *(38)* |
| Adiponectin | Enhancement | Serum adiponectin is low in obese humans *(37)* and increases following weight loss *(39)* | Adipocytes *(37)* | Higher in VF of rodents *(38)* |
| Visfatin | Enhancement | Increased in human visceral adiposity *(64)* | Adipocytes *(68)* | Higher in VF of humans *(66)* |
| Leptin | Enhancement | High in obese humans, because of leptin resistance | Adipocytes *(2)* | Higher in SCF of humans *(8)* |

## VASPIN:

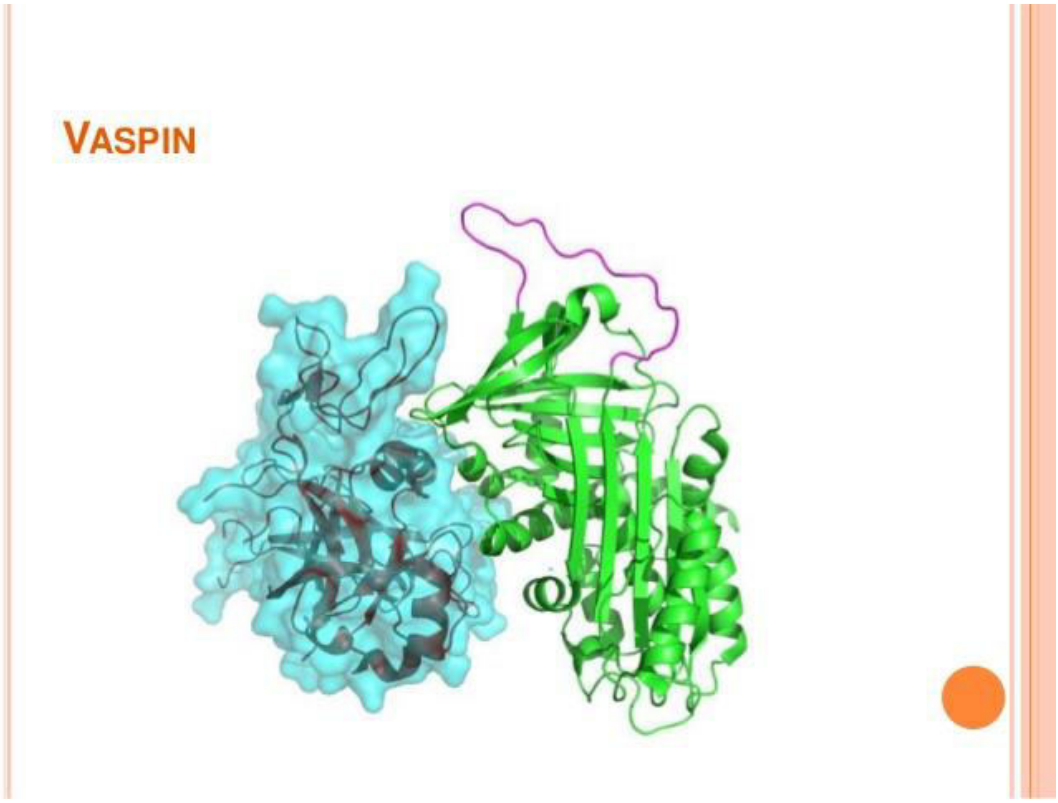
**Vaspin** (Visceral adipose tissue derived serpin A12 ) is an adipokine that has been identified as a member of serine protease inhibitor family, which is predominantly secreted in the visceral white adipose tissue of "Otsuka Long Evans Tokushima fatty (OLETF) rats", which are an animal model of obesity and Type 2 DM[123].

**Figure 22. Otsuka Long Evans Tokushima fatty ( OLETF ) rats :**


**Vaspin** is also found to be expressed in the skin, hypothalamus, pancreatic islets and stomach and the liver and a significantly higher expression was found from the visceral adipose tissues when compared to the subcutaneous adipose tissues. Human **Vaspin** protein consists of 415 amino acids and homology analysis indicate that **vaspin** has approximately about 40% identity with alpha 1 antitrypsin [124].

The serum **vaspin** levels were found to be highest at an age when obesity and the plasma insulin concentrations reached a peak in these OLETF rats and the levels markedly decreased when they developed severe hyperglycemia and the levels were normalized after treatment with insulin or insulin sensitizing agents like pioglitazone as studied by **Nida et al., 2005**[125].

**Figure 23. Structure of vaspin :**




Matthias Bluher et al in the year 2011 put forward that the serum vaspin levels were also found to be increased along with an increased vaspin mRNA expression in human adipose tissue in obese individuals with insulin resistance and type 2 DM and the lean human individuals had an undetectable vaspin mRNA expression [225].

**Mechanism of action of Vaspin:**

Serpins inhibit serine proteases by a unique suicide mechanism. They contain an exposed reactive centre loop (RCL) that is presented to the target protease as a pseudosubstrate. The amino acid sequence of the RCL determines which serine protease will be inhibited by the serpin.

Binding of the protease to the RCL induces conformational changes of the serpin which thus deforms the reactive centre of the protease and inactivates it[126].

**Vaspin** inhibits a protease which plays a role in the degradation of a hormone or molecule which has a direct or indirect glucose lowering effects.

The increased levels of **vaspin** in obese individuals are due to a compensatory phenomenon and mechanism in response to the decreased insulin sensitivity (insulin resistant state) or impairment of glucose metabolism. (i.e) an increase in the **vaspin** concentrations is due to a compensatory response to antagonise the action of the other unknown proteases that are up regulated in obesity and in states of insulin resistance. Thus it serves to be a protective and defensive mechanism aimed to reduce insulin resistance in humans[127, 128].

Also the compensatory capacities of serum **vaspin** concentrations were found to decline with the progression of the obesity related metabolic dysfunction and thus its protective mechanism might be lost with the development of complications of obesity [129].

A central administration of **vaspin** to obese mice also improved their glucose tolerance, insulin sensitivity, affected the gene expression of candidate genes for insulin resistance and reduced the food intake and this favourable effect of vaspin results in bringing the plasma glucose levels to normal and modifying the expression of genes involved in the pathogenesis of insulin resistance. Thus **vaspin** serves as an insulin sensitizer [130].

Thus identification of the target proteases which are inhibited by **vaspin** would lead to the development of new strategies in the treatment of obesity and would help in preventing the occurance of its metabolic complications.

Human Kallikrein (hk7) has been found as the first serine protease which is inhibited by Vaspin. Insulin was found to be a substrate of hK7. The inhibition of hk7 by Vaspin prevents a hk7 mediated insulin degradation and stabilizing the circulating Insulin concentrations – thus improving the glycemic control in patients with Type 2 DM [131].

# AIM

# &

# OBJECTIVES

# AIM & OBJECTIVES

AIM:

To determine the circulating Serum Vaspin levels in humans with obesity in order to assess its association and link to obesity related metabolic alterations.

OBJECTIVES:

1. To estimate the circulating Serum Vaspin levels in humans with obesity and in healthy control subjects.

2. To estimate the anthropometric measurements (i.e the standing height & weight), the measures of obesity (i.e the Waist and Hip Circumference, the Waist/Hip ratio and the BMI) in the humans with obesity and in the healthy control subjects.

3. To estimate the Lipid profile, the Fasting Blood Glucose levels, the Fasting serum insulin levels and the Insulin resistance by the HOMA-IR method in the humans with obesity and in the healthy control subjects.

4. To assess and compare the Serum Vaspin levels and its correlation with the above said parameters in the humans with obesity and in the healthy control subjects.

# MATERIALS AND METHODS

The study was conducted during the period of June 2016 to May 2017 at the Institute of Physiology and Experimental Medicine, Madras Medical College, Institute of Internal Medicine and The Medical Endocrine Clinic, Rajiv Gandhi Government General Hospital after obtaining approval from Institutional Ethics Committee (IEC), Madras Medical College, Chennai-3.

## Selection of subjects:

Thirty obese subjects in the age group of 30 to 55 years having a BMI of ≥ 35 (Group I ) and thirty subjects of the same age group with a normal range BMI ( Group II ) were selected from the Institute of Internal Medicine and The Medical Endocrine Clinic, Rajiv Gandhi Government General Hospital for the study.

## Selection of the Sample Size:

For this study, a minimum sample size of 30 per group was required to have an 85% chance (alpha error 0.05). Therefore a sample size of 60 was arrived at.

## Inclusion criteria:

Subjects in the age group of 30 to 55 years having a BMI of ≥ 35 and thirty subjects of the same age group with a normal range BMI were included in the study.

**Exclusion criteria:**

Subjects with:

1. Age < 30 and > 55 years

2. BMI < 35 and > 45 kg/m

3. Type I and Type II DM [according to the American Diabetes Association Criteria]

4. Renal / Hepatic disease

5. Acute/ Chronic Inflammatory disease [as determined by a Leukocyte count of >7000cells/cu mm

6. H/O hypertension [SBP < 140 mmHg and DBP < 85 mmHg]

7. Thyroid dysfunction/PCOS and Cushing's disease

8. Ischemic Cardiovascular disease

9. Alcohol or drug abuse/ Smoking

10. Cancer

11. Acute or Chronic infections

12. Any haematological disorder

13. Hormonal therapy

14. Chronic Medication therapies (such as on antidepressants, anticonvulsants, hypoglycaemic drugs, anti hypertensives, lipid lowering agents, OCPs and corticosteroids)

15. Pregnancy

16. Any chronic medical or psychiatric illness

**STUDY DESIGN:** Cross Sectional Study

**PLACE OF STUDY:**

1. Institute of Physiology & Experimental Medicine,

   Madras Medical College, Chennai-3.

2. Institute of Internal Medicine,

   Rajiv Gandhi Government General Hospital, Ch-3.

3. Medical Endocrine clinic

   Rajiv Gandhi Government General Hospital, Ch-3.

**Methodology:**

Thirty obese subjects in the age group of 30 to 55 years having a BMI of ≥ 35 (Group I) and thirty subjects of the same age group with a normal range BMI (Group II) participated in the study.

After obtaining an informed consent and before inclusion in the study, a detailed history was obtained from all the study subjects and they also underwent a careful and through physical examination and laboratory investigations to exclude any condition that might interfere with the study parameters.

The following were obtained and measured for all the study subjects using standard protocols:

1. The anthropometric measurements ( i.e) the standing height and weight in light clothing without shoes were obtained using a stretch resistant measuring tape and the BMI was calculated using the formula wt (kg) / Ht ($m^2$).

2. The measures of obesity (i.e) the waist circumference, the hip circumference were obtained and the waist hip ratio was calculated.

According to the WHO Stepwise Approach to surveillance (STEPS) protocol, the measurement for the waist circumference was made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration. The measurement was made with a stretch resistant measuring tape that provides a constant 100gm of tension, with the tape parallel to the floor.

According to the WHO Stepwise Approach to surveillance (STEPS) protocol, the measurement for the hip circumference was taken around the widest portion of the buttocks.

It was ensured that the subject was standing erect with relaxed abdominal muscles, the arms by the side, feet positioned close together and the weight evenly distributed across the feet and the clothing removed from the waistline. The measurement was made with a stretch resistant measuring tape that provides a constant 100gm of tension, with the tape parallel to the floor.

3. After an overnight fast, between 8 and 10 am a blood sample was taken and serum collected and stored at - 80°C.
4. The fasting blood glucose levels, the lipid profile (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol), the fasting serum insulin levels were obtained.

5. The insulin resistance was estimated by the Homeostasis model assessment method (HOMA – IR) by using the formula fasting insulin (μIU/L) x fasting glucose (mg/dL)/405.

6. Serum vaspin levels were assayed using the commercially available human vaspin ELISA kit using a human vaspin sandwich ELISA technique.

The sample handling, storage and preparation were done according to the manufacturer's instructions.

The serum vaspin levels of the obese subjects were compared and correlated with the subjects who had a normal BMI.

**ESTIMATION OF THE FASTING BLOOD SUGAR LEVELS**:

**Sample Collection and Storage**:

- Under aseptic precautions about 5ml of a fasting blood sample was collected from the subject and was centrifuged and serum separated.
- The serum samples were stored at -20 degrees Celsius.
- Roche Diagnostics Cobas GLU HK Gen.3 kit which contains an in vitro diagnostic reagent system was used for estimating the serum fasting blood glucose levels by the Electrochemiluminescence method. The test was done at the Central lab, Institute of Biochemistry, Madras Medical College, Chennai.

**Principle of the test:**

- In electro generated chemiluminescence, electrochemically generated intermediates undergo a highly exergonic reaction to produce an electronically excited state that then emits light.
- It is an enzymatic reference method with hexokinase.
- Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.
- In the presence of NADP, Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

**Reagents Required:**

- R1: MES buffer: 5.0 mmol/L, pH 6.0; Mg2+: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative.
- R2:HEPES buffer: 200 mmol/L, pH 8.0; Mg2+: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G-6-PDH (E. coli): ≥ 300 µkat/L; preservative.

**Assay Procedure:**

After calibration and controls had been measured, the test samples were loaded on to the Cobas Analyser and the desired test was ordered in the host computer system. The samples were evaluated for the fasting blood glucose levels and when the analysis was complete, the results were automatically obtained.

**ESTIMATION OF FASTING LIPID PROFILE LEVELS:**

**Sample Collection and Storage**:

- Under aseptic precautions about 5ml of a fasting blood sample was collected from the subject and was centrifuged and serum separated.
- The serum samples were stored at -20 degrees Celsius.
- Roche Diagnostics Cobas CHOL Gen.2, HDLC Gen.3 and TRIGL kits were used for estimating the serum total cholesterol, HDL and triglycerides by the Electrochemiluminescence method. The test was done at the Central lab, Institute of Biochemistry, Madras Medical College, Chennai.

**Principle of the test:**

**For estimation of Total Cholesterol:**

- In electro generated chemiluminescence, the intermediates which are electrochemiccaly generated undergo an exergonic reaction and produce an electronically excited state that emits light.
- It is an enzymatic, colorimetric method.
- Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.
- Cholesterol oxidase catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide.

- In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye.
- The cholesterol concentration is directly proportional to the colour intensity of the dye that is formed and this is determined by measuring the increase in absorbance.

**For the estimation of HDL:**

- In electrogenerated chemiluminescence, The intermediates undergo a highly exergonic reaction in order to produce an electronically excited state that then emits light.
- It is a homogeneous enzymatic colorimetric test.
- In the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymes.
- The HDL-cholesterol concentartion is determined by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups enzymatically. (approx. 40 %).
- Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.
- In the presence of oxygen, cholesterol oxidase oxidises cholesterol to Δ4-cholestenone and hydrogen peroxide.

- In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye, whose colour intensity is directly proportional to the cholesterol concentration. This is then measured photoelectrically.

**For the estimation of triglycerides:**

- In electrogenerated chemiluminescence, intermediates undergo an exergonic reaction in order to produce an electronically excited state that then emits light.
- It is an enzymatic colorimetric test.
- This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide.
- Under the catalytic action of peroxidise, the hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol to form a red dyestuff (Trinder endpoint reaction).
- The color intensity of the red dye stuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

**Reagents Required:**

**For the estimation of Total Cholesterol:**

- R1 : PIPES buffer: 225 mmol/L, pH 6.8; Mg2+: 10 mmol/L; sodium cholate:0.6 mmol/L; 4-aminophenazone: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L;fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonasspec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L(≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL);stabilizers; preservative.

**For the estimation of HDL:**

- R 1: HEPES buffer: 10.07 mmol/L; CHES 96.95 mmol/L, pH 7.4; dextransulfate: 1.5 g/L; magnesium nitrate hexahydrate: > 11.7 mmol/L; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium sp., recombinant):> 50 µkat/L; peroxidase (horseradish): > 16.7 µkat/L; preservative.
- R2: HEPES buffer: 10.07 mmol/L, pH 7.0; PEG-cholesterol esterase (Pseudonomas spec.): > 3.33 µkat/L; PEG-cholesterol oxidase (Streptomyces sp., recombinant): > 127 µkat/L; peroxidase (horseradish): > 333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L; preservative.

**For the estimation of triglycerides:**

- R1: PIPES buffer: 50 mmol/L, pH 6.8; Mg2+: 40 mmol/L; sodium cholate:0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L;4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.):≥ 83 μkat/L; glycerokinase (Bacillus stearothermophilus): ≥ 3 μkat/L;glycerol phosphate oxidase (E. coli): ≥ 41 μkat/L; peroxidase (horseradish):≥ 1.6 μkat/L; preservative.

**Assay Procedure:**

After calibration and controls had been measured, the test samples were loaded on to the Cobas Analyser and the desired test was ordered in the host computer system. The samples were evaluated for the fasting lipid profile and when the analysis was complete, the results were automatically obtained.

**Estimation of the fasting Serum Insulin Levels:**

**Sample Collection and Storage**:

- Under aseptic precautions about 5ml of a fasting blood sample was collected from the subject and was centrifuged and serum separated.
- The serum samples were stored at -20 degrees Celsius.
- Roche Diagnostics Insulin kit was used for estimating the serum fasting Insulin levels by the Electrochemiluminescence method. The test was done at the Central lab, Institute of Biochemistry, Madras Medical College, Chennai.

**Test Principle:**

- Sandwich principle. Total duration of assay: 18 minutes.
- 1st incubation: Insulin from 20 µL sample, a biotinylated monoclonal insulin‑ specific antibody, and a monoclonal insulin‑ specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: The complex becomes bound to the solid phase via interaction of biotin and streptavidin after addition of streptavidin-coated microparticles, .
- The reaction mixture obtained is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve. This is instrument specifically generated by 2‑ point calibration and a master curve provided via the reagent barcode.

**Reagents Required:**

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-insulin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti‑ insulin antibody (mouse) 1 mg/L; MESb) buffer 50 mmol/L, pH 6.0; preservative.

- R2 Anti-insulin-Ab~Ru(bpy) (black cap), 1 bottle, 10 mL:

  Monoclonal anti‑ insulin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.

**Assay Procedure:**

After calibration and controls had been measured, the test samples were loaded on to the Cobas Analyser and the desired test was ordered in the host computer system. The samples were evaluated for the fasting serum insulin levels and when the analysis was complete, the results were automatically obtained.

**Estimation of Serum Vaspin Levels:**

**Sample Collection and Storage**:

- Under aseptic precautions about 5ml of a fasting blood sample was collected from the subject and was centrifuged and serum separated.
- The serum samples were stored at -20 degrees Celsius.
- Elabscience Vaspin Elisa kit was used for estimating the serum fasting blood glucose levels by the Electrochemiluminescence method. The test was done at the Central lab, Institute of Biochemistry, Madras Medical College, Chennai.

**Test Principle:**

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to VASPIN. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody.

Then an antibody specific for VASPIN and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain VASPIN, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectro photometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of VASPIN. You can calculate the concentration of VASPIN in the samples by comparing the OD of the samples to the standard curve.

**Reagents Required:**

1. Reference Standard
2. Reference Standard & Sample Diluent
3. Concentrated Biotinylated Detection Ab
4. Biotinylated Detection Ab Diluent
5. Concentrated HRP Conjugate
6. HRP Conjugate Diluent
7. Concentrated Wash Buffer
8. Substrate Reagent
9. Stop solution

**Other Materials Required:**

1. Micro Elisa Plate
2. Microplate reader with 450nm wavelength filter
3. High-precision transferpettor, EP tubes and disposable pipette tips
4. 37℃ Incubator
5. Deionized or distilled water
6. Absorbent paper
7. Loading slot for Wash Buffer
8. Plate Sealer

**Assay Procedure:**

All reagents and samples were brought to room temperature before use. The sample was centrifuged again after thawing before the assay.

All the reagents were mixed thoroughly before pipetting and it was taken care that foaming was avoided.

1. 100µLof Standard and Samples were added to each well. The blank well was added with Reference Standard & Sample diluent. Solutions were added to the bottom of micro ELISA plate well and were mixed gently and the plate was covered with sealer and was incubated for 90 minutes at 37℃.

2. The liquid of each well was removed and 100µL of Biotinylated Detection Ab working solution was added to each well. It was then covered with the Plate sealer which was then incubated for 1 hour at 37°C.

3. Each well was aspirated and washed, and this process was repeated three times. Then wash was done by filling each well with Wash Buffer (approximately 350µL) Complete removal of liquid at each step was ensured. After the last wash, the remaining Wash Buffer was removed by aspirating and the plate was inverted and patted against thick clean absorbent paper.

4. 100µL of HRP Conjugate working solution was added to each well and covered with the Plate sealer which was then incubated for 30 minutes at 37°C.

5. The wash process was repeated for five times as conducted in step 3.

6. 90µL of Substrate Solution was added to each well and covered with a new Plate sealer which was then incubated for about 15 minutes at 37°C. When apparent gradient appeared in standard wells, the reaction was terminated.

7. 50µLof Stop Solution was added to each well and the colour turned yellow immediately.

Using a micro-plate reader set to 450 nm, the optical density of each well was determined.

**Calculation of Results:**

Absorbance (Optical density) values of standard controls and samples were calculated. With concentration on X axis and absorbance value on Y axis,

plotting the mean absorbance got from each standard against its concentration, a standard curve was constructed. Using this standard curve based on mean absorbance value, corresponding concentration of each sample was found.

The data was analyzed, using the IBM SPSS software version 22.

**Normal Values**:

Fasting Blood sugar – 70 to 110 mg/dl

Total cholesterol < 200mg/dl

HDL – 35.3 to 79.5 mg/dl

LDL < 100mg/dl

TGL – 40 to 160mg/dl

Serum Insulin – 2 to 25 µIU/mL

HOMA – IR – < 3

The minimum detectable range of Serum Vaspin using the Elabscience Vaspin Elisa kit is 0.0625 to 4 ng/ml.

# RESULTS

# RESULTS

A total of 60 subjects were included in the study.

**Table 7: Descriptive analysis of Gender in Group I (obese subjects) (N = 30):**

| Gender | Frequency | Percentage |
|---|---|---|
| Male | 5 | 16.66% |
| Female | 25 | 83.33% |

**Table 8: Descriptive analysis of Gender in Group II (Non - obese subjects) (N = 30):**

| Gender | Frequency | Percentage |
|---|---|---|
| Male | 3 | 10% |
| Female | 27 | 90% |

**Table 9 : Descriptive measurements of the study participants:**

| VARIABLES | GROUP I (Obese subjects)<br><br>Mean ± SD | GROUP II ( Non – Obese)<br><br>Mean ± SD |
|---|---|---|
| BMI | 40.59± 3.98 | 22.87± 1.81 |
| Waist Circumference (cms) | 115± 9.13 | 83.23± 1.54 |
| Hip Circumference(cms) | 127.47± 10.75 | 104.60± 3.45 |
| W/H Ratio | 0.95± 0.073 | 0.79± 0.02 |
| FBS ( mg/dl) | 90.6 ± 18.123 | 62.07 ± 15.04 |
| TC (mg/dl) | 186.23 ± 51.54 | 145.27 ± 33.77 |
| HDL (mg/dl) | 44.9 ± 1.98 | 47.5 ± 2.78 |
| TGL (mg/dl) | 136.8 ± 43.21 | 106.07 ± 70.21 |
| TC/HDL Ratio | 4.89 ± 1.86 | 4 ± 1.33 |
| LDL/HDL Ratio | 3.02 ± 1.58 | 2 ± 0.87 |
| Serum Insulin<br><br>(µIU/mL) | 29.21 ± 20.17 | 11.05 ± 6.53 |
| Insulin Resistance | 6.60 ± 4.41 | 1.81 ± 1.59 |
| Serum Vaspin<br><br>(ng/ml) | 1.26 ± 1.18 | 0.73± 0.59 |

The statistical analysis of the data was performed using the IBM SPSS software version 22.The Mean and Standard deviation of the variables were determined for the two study groups. The Unpaired student t test was employed as the Test of significance at 95% confidence interval.

*** P value < 0.05 was considered as statistically significant**

**** P value < 0.01 was considered as highly statistically significant**

*****P value < 0.001 was considered as very highly statistically significant**

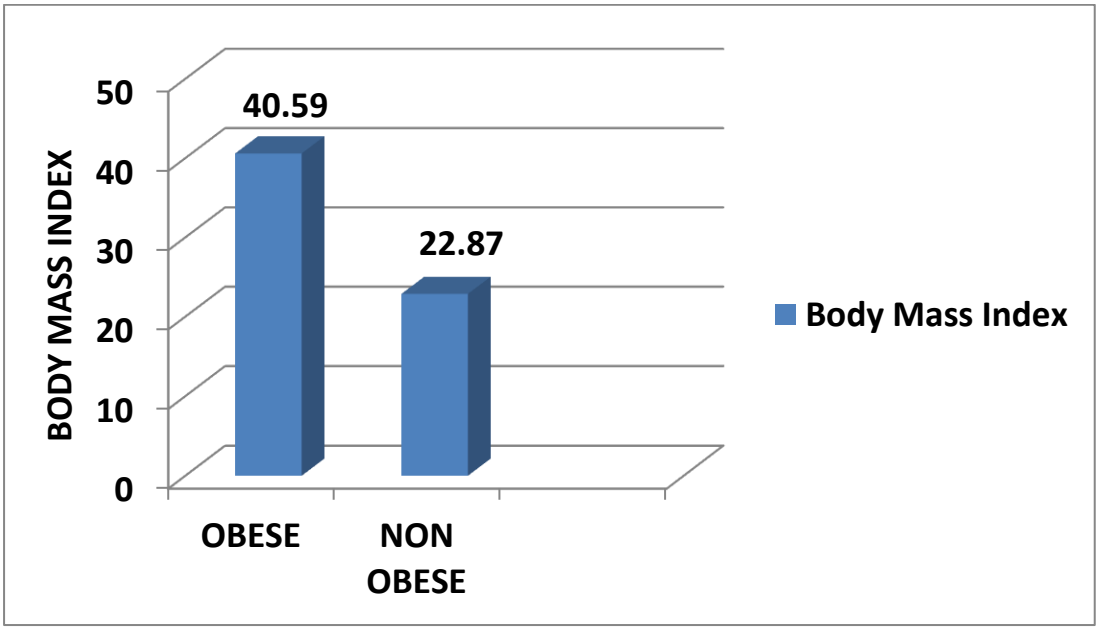
## TABLE 10

## COMPARISON OF THE BMI BETWEEN THE TWO STUDY GROUPS

| VARIABLES | STUDY GROUPS | MEAN | SD | P Value |
|---|---|---|---|---|
| BMI (kg/m²) | GROUP I | 40.59 | 3.98 | 0.0001*** |
| | GROUP II | 22.87 | 1.81 | |

Table No.10 compares the age and BMI of the two study groups. The P values of the age and BMI were very highly significant.

**Graph 1: COMPARISON OF THE BMI BETWEEN THE TWO STUDY GROUPS**



## TABLE 11

## COMPARISON OF THE MEASURES OF OBESITY (WAIST CIRCUMFERENCE, HIP CIRCUMFERENCE AND WAIST HIP RATIO) BETWEEN THE TWO STUDY GROUPS

| VARIABLES | STUDY GROUPS | MEAN | SD | P Value |
|---|---|---|---|---|
| WAIST CIRCUMFERENCE (cms) | GROUP I | 115.5 | 9.13 | 0.0001*** |
| | GROUP II | 83.23 | 1.54 | |
| HIP CIRCUMFERENCE (cms) | GROUP I | 127.47 | 10.75 | 0.0001*** |
| | GROUP II | 104.60 | 3.45 | |
| WAIST/ HIP RATIO | GROUP I | 0.95 | 0.073 | 0.0001*** |
| | GROUP II | 0.79 | 0.02 | |

Table No.11 compares the waist circumference, hip circumference and the waist/ hip ratio between the two study groups.

The P values of waist circumference, the hip circumference and the waist/hip ratio were very highly significant.

**Graph 2: COMPARISON OF THE MEASURES OF OBESITY (WAIST CIRCUMFERENCE, HIP CIRCUMFERENCE AND WAIST HIP RATIO) BETWEEN THE TWO STUDY GROUPS**

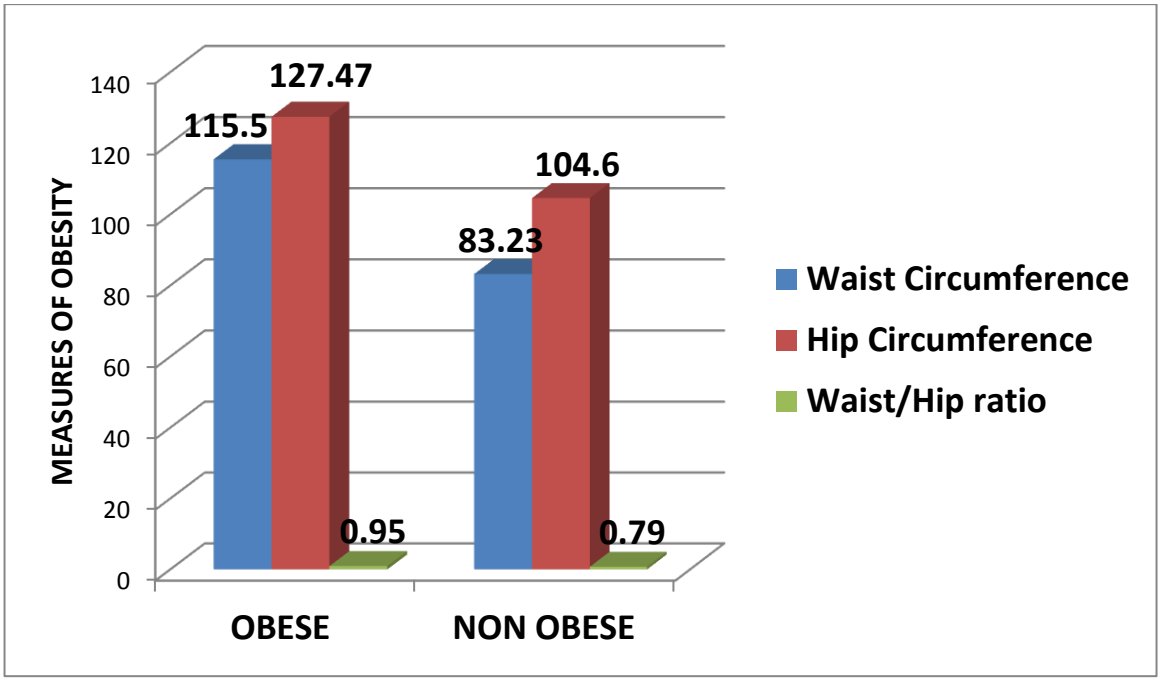


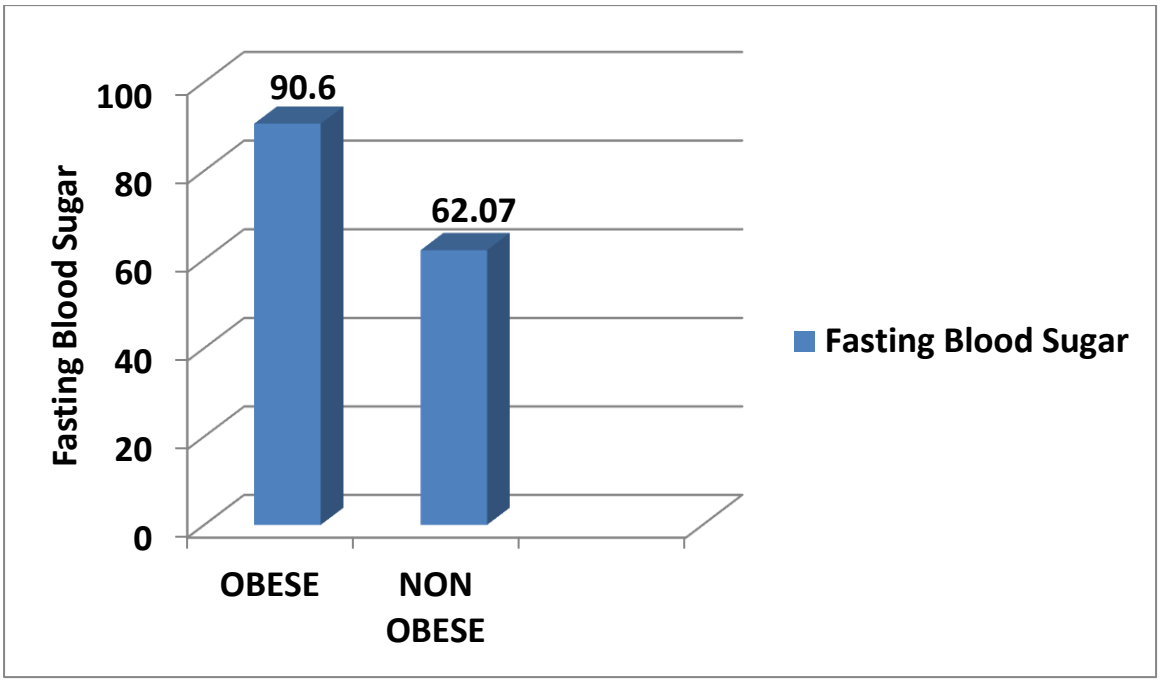

**TABLE 12**

**COMPARISON OF THE SERUM FASTING BLOOD SUGAR LEVELS BETWEEN THE TWO STUDY GROUPS**

| VARIABLES | STUDY GROUPS | MEAN | SD | P Value |
|---|---|---|---|---|
| SERUM FBS (mg/dl) | GROUP I | 90.6 | 18.12 | 0.0001*** |
| | GROUP II | 62.07 | 15.04 | |

Table No.12 compares the serum fasting blood sugar levels between the two study groups .The P value of the serum fasting blood sugar levels was very highly significant.

**Graph 3: COMPARISON OF THE SERUM FASTING BLOOD SUGAR LEVELS BETWEEN THE TWO STUDY GROUPS**



## TABLE 13

## COMPARISON OF THE FASTING LIPID PROFILE BETWEEN THE TWO STUDY GROUPS

| VARIABLES | STUDY GROUPS | MEAN | SD | P Value |
|---|---|---|---|---|
| TOTAL CHOLESTEROL (mg/dl) | GROUP I | 186.23 | 51.54 | 0.0006*** |
| | GROUP II | 145.27 | 33.77 | |
| HDL (mg/dl) | GROUP I | 44.9 | 1.98 | 0.0001*** |
| | GROUP II | 47.5 | 2.78 | |
| LDL (mg/dl) | GROUP I | 115 | 48.16 | 0.0175* |
| | GROUP II | 90.63 | 25.63 | |
| TGL (mg/dl) | GROUP I | 136.8 | 43.21 | 0.0457* |
| | GROUP II | 106.07 | 70.21 | |
| TC/HDL | GROUP I | 4.89 | 1.86 | 0.0373* |
| | GROUP II | 4 | 1.33 | |
| LDL/HDL | GROUP I | 3.02 | 1.58 | 0.0030** |
| | GROUP II | 2 | 0.87 | |

Table No.13 compares the fasting lipid profile between the two study groups.

The P values of total cholesterol and HDL were very highly significant.

The P values of LDL, triglycerides and TC/HDL ratio were significant.

The P value of LDL/HDL ratio was highly significant.

**Graph 4: COMPARISON OF THE FASTING LIPID PROFILE BETWEEN THE TWO STUDY GROUP**

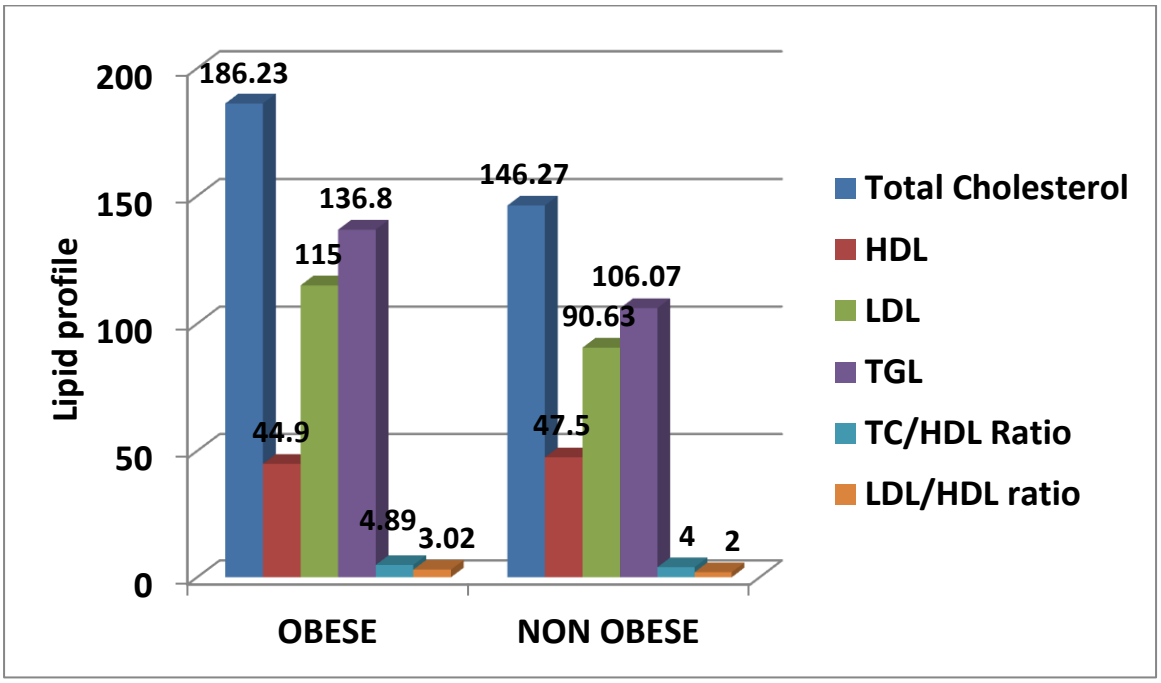



**TABLE 14**

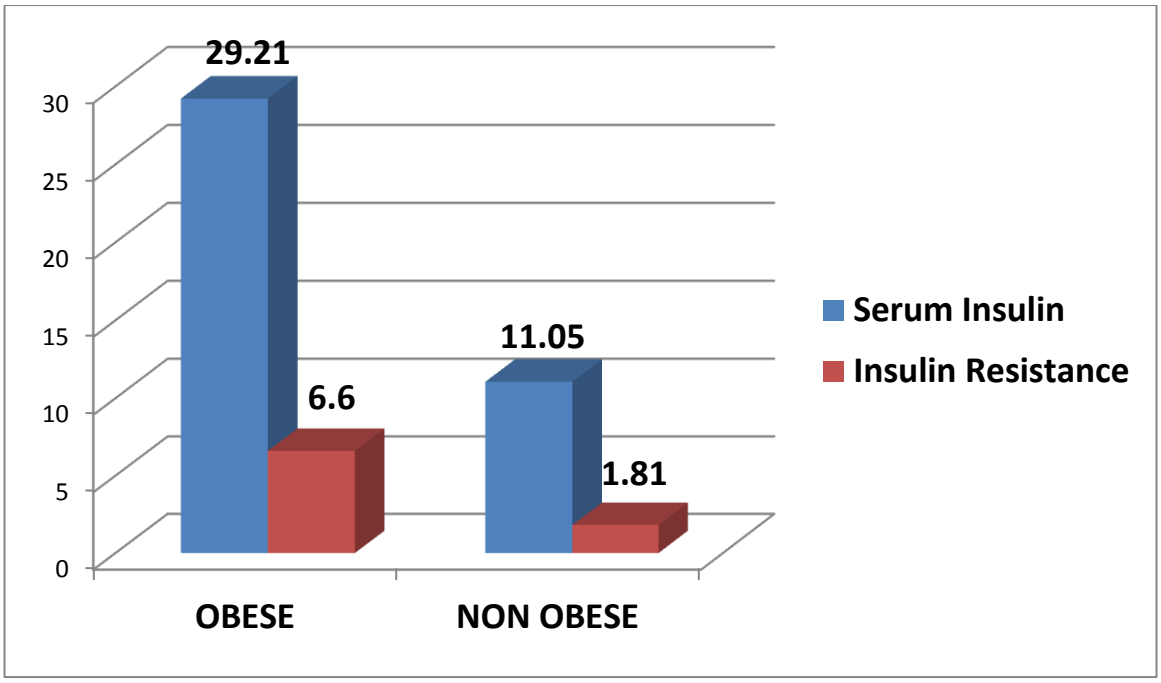
**COMPARISON OF THE SERUM FASTING INSULIN LEVELS AND INSULIN RESISTANCE BETWEEN THE TWO STUDY GROUPS**

| VARIABLES | STUDY GROUPS | MEAN | SD | P Value |
|---|---|---|---|---|
| SERUM INSULIN (µIU/mL) | GROUP I | 29.21 | 20.17 | 0.0001*** |
| | GROUP II | 11.05 | 6.53 | |
| INSULIN RESISTANCE | GROUP I | 6.60 | 4.41 | 0.0001*** |
| | GROUP II | 1.81 | 1.59 | |

Table No.14 compares the Serum fasting insulin levels and Insulin resistance of the two study groups.

The P values of the Serum fasting insulin levels and Insulin resistance were very highly significant.

**Graph 5: COMPARISON OF THE SERUM FASTING INSULIN LEVELS AND INSULIN RESISTANCE BETWEEN THE TWO STUDY GROUPS**



## TABLE 15

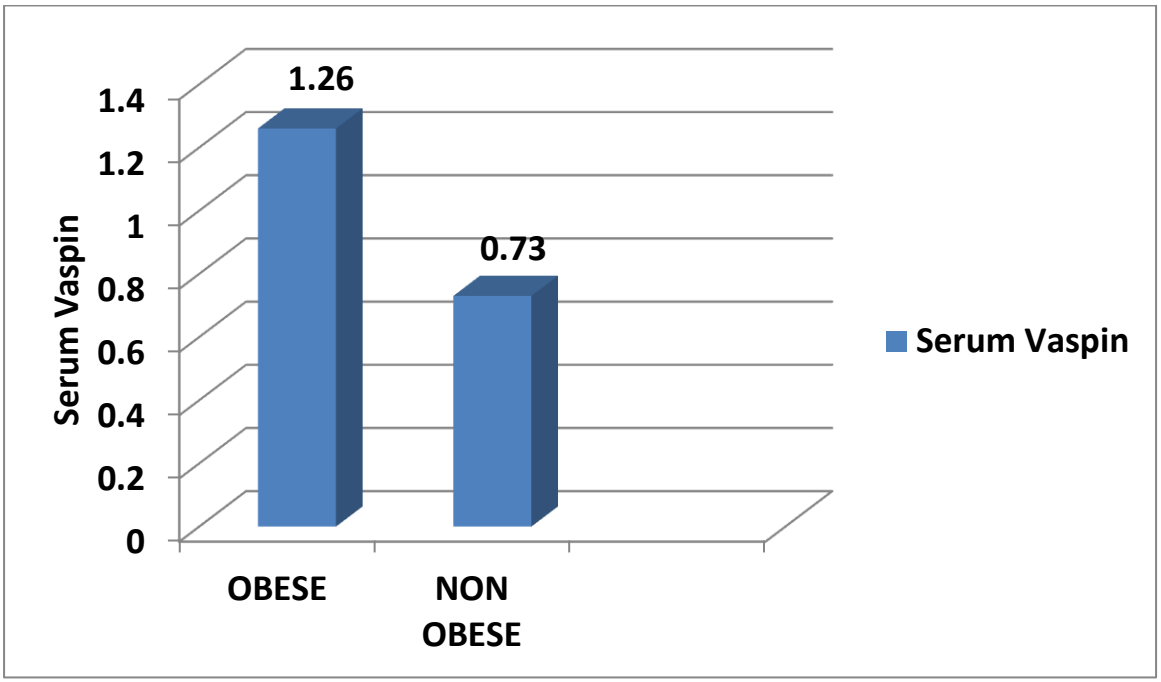
## COMPARISON OF THE SERUM VASPIN LEVELS BETWEEN THE TWO STUDY GROUPS

| VARIABLES | STUDY GROUPS | MEAN | SD | P Value |
|---|---|---|---|---|
| SERUM VASPIN (ng/ml) | GROUP I | 1.26 | 1.18 | 0.0318* |
| | GROUP II | 0.73 | 0.59 | |

Table No.15 compares the serum vaspin levels between the two study groups.

The P value of the serum vaspin was statistically significant.

**Graph 6: COMPARISON OF THE SERUM VASPIN LEVELS BETWEEN THE TWO STUDY GROUPS**



## Table 16

## CORRELATION OF SERUM VASPIN LEVELS WITH THE AGE AND BMI

| VARIABLE | | AGE ( YEARS) | BMI ( kg/m²) |
|---|---|---|---|
| VASPIN | r | 0.115 | 0.722 |
| | P | 0.54 | 0.00001*** |

## Table 17

## CORRELATION OF SERUM VASPIN LEVELS WITH THE WAIST CIRCUMFERENCE, HIP CIRCUMFERENCE AND WAIST HIP RATIO

| VARIABLE | | WC (cms) | HC (cms) | W/H RATIO |
|---|---|---|---|---|
| VASPIN | r | 0.6140 | 0.5775 | -0.0815 |
| | P | 0.0003*** | 0.0008*** | 0.67 |

## Table 18

## CORRELATION OF SERUM VASPIN LEVELS WITH THE SERUM FASTING BLOOD SUGAR LEVELS

| VARIABLE | | FBS (mg/dl) |
|---|---|---|
| VASPIN | R | 0.0802 |
| | P | 0.767 |

## Table 19

## CORRELATION OF SERUM VASPIN LEVELS WITH THE LIPID PROFILE

| VARIABLE | | TC(mg/dl) | HDL(mg/dl) | LDL(mg/dl) | TGL | TC/HDL | LDL/HDL |
|---|---|---|---|---|---|---|---|
| VASPIN | r | -0.245 | -0.35 | 0.254 | 0.0708 | 0.004 | 0.052 |
| | P | 0.19 | 0.168 | 0.174 | 0.7 | 0.97 | 0.78 |

## Table 20

## CORRELATION OF SERUM VASPIN LEVELS WITH THE FASTING SERUM INSULIN LEVELS AND INSULIN RESISTANCE

| VARIABLE | | SERUM INSULIN ( µIU/ml) | INSULIN RESISTANCE |
|---|---|---|---|
| VASPIN | R | 0.070 | 0.139 |
| | P | 0.595 | 0.289 |

Pearson's correlation revealed that serum vaspin levels were positively associated with the age, BMI, waist circumference, hip circumference HDL, LDL, TGL, TC/HDL ratio, LDL/HDL ratio, the fasting blood sugar levels, serum insulin levels and insulin resistance.

The serum vaspin levels were negatively correlated with the waist hip ratio, fasting blood sugar and total cholesterol levels.

(** Pearson's correlation is significant at the level $P < 0.05$)

# DISCUSSION

# DISCUSSION

Obesity is a common chronic disorder of excessive body fat and has become a global epidemic which is prevalent in the industrialised, developing and in the underdeveloped countries.

Obesity increases the risk of developing a variety of adverse consequences to human health ranging from metabolic disturbances including type 2 Diabetes Mellitus and cardiovascular complications to disorders of the locomotor system and many types of cancer[132].

Increased visceral adiposity is usually associated with a clustering of atherogenic risk factors and it plays a central role in insulin resistance and its related metabolic alterations. Adipokines form a new exciting link between obesity and insulin resistance[133].

In the current study, the purpose was to estimate and investigate the role of vaspin as a novel potential biomarker of insulin resistance in the obese subjects. This was with the view that serum vaspin levels could be used as an early identification of the obesity and its related metabolic alterations, which would in turn enable us to make early interventions to protect oneself from its ruinous complications.

In my study, the BMI, measures of obesity, the lipid profile, serum insulin levels, the insulin resistance and the serum vaspin levels were estimated and compared in both the obese and the non obese subjects.

And the serum vaspin levels were also correlated with the measures of obesity and the insulin resistance in both the obese and the non obese subjects.

In this study, the obese subjects showed an elevated BMI when compared to the non obese subjects. The vaspin levels also showed a significant positive correlation with their BMI values.

This means that the vaspin concentration increases with an increase in BMI.

The results presented here are in line with **Youn et al., (2008)**[134] and **Esaki et al., (2009)**[135] who observed a significant BMI adjusted correlation with vaspin.

Regarding the measures of obesity, the waist circumference, the hip circumference and the waist hip ratio were found to be elevated in the obese subjects when compared to the non obese subjects with a normal range BMI.

And the waist circumference of both the obese and the non obese subjects showed a significant positive correlation with their serum vaspin levels. This result is in accordance to **Scott M Grundy et al., (2013)**[136] who proposed that the waist circumference is a globally used parameter to quantify central obesity and is the key culprit in insulin resistance and its related complications. Thus it can be proposed that greater the waist circumference of an individual, greater would be the serum vaspin levels. This finding in my study is to be highlighted as it suggests that a higher serum vaspin levels in the obese individuals is an indicator of visceral obesity and its related complications.

Looking at the lipid profile in this study, the total cholesterol, the serum triglyceride levels and the LDL cholesterol showed a significant elevation in the obese subjects when compared to the non obese subjects. The TGL levels and the HDL cholesterol levels, LDL cholesterol levels and the cardiovascular risk assessment ratios showed a positive with the serum vaspin levels.

These results agree with **El-Mesallamy et al.,(2011)**[137] who demonstrated that vaspin levels are significantly correlated with markers of lipid metabolism such as TGL and to a lesser extent LDL cholesterol, indicating that vaspin may play a role in lipid metabolism or might be induced as a compensatory response by dyslipidemia in visceral obesity. This is especially because vaspin is an adipokine secreted by adipocytes.

This observation is also in accordance with **Pascot et al., (2001)**[138] and **Boudenwijri Klop et al., (2013)**[139] who reported that hypertriglyceridemia and the dyslipidemic state (characterised by an increased fasting triglycerides, high LDL cholesterol, low HDL cholesterol, elevated blood glucose and insulin levels) found in subjects with a visceral obesity was an independent and a key contributing factor in increasing the risk for cardiovascular disorders.

Thus this positive association suggests that increased serum Vaspin levels in obese individuals increases their risk of acquiring a cardiovascular disease.

Furthermore evaluating the CVD risk assessment ratios, TC/HDL- C and LDL- C/HDL- C, a significant difference was found in the obese and the non obese subjects.

This study also demonstrated that the serum vaspin levels and the insulin resistance of the obese subjects were significantly elevated when compared to the non obese subjects. And the serum insulin levels and the insulin resistance in both the obese and the non obese subjects showed a positive correlation with their serum vaspin levels.

These results suggest that the obese subjects with a high serum vaspin levels may be prone to develop the obesity related metabolic complications in the future.

It was postulated that an increase in the serum vaspin levels in the obese individuals was due to an increased secretion of the adipokine vaspin from the visceral adipose tissues and represented a compensatory mechanism or a response associated with obesity in order to antagonize the action of the other unknown proteases that are up regulated in obesity and in states of insulin resistance. Hence this up regulation is said to be a defensive mechanism against insulin resistance **(Hida et al., 2005 ; Kloting et al., 2006; Zvonic et al., 2007)**[140,141& 142].

In addition the results showed that there is a positive correlation between age and vaspin concentration in the obese subjects.

These results are in agreement with **Youn et al.,(2008)**[143] who reported that age is an independent predictor of vaspin concentration in obese subjects.

There is also a positive correlation of the fasting blood sugar levels and the serum vaspin levels in the obese subjects.

These results are in agreement with **El-Mesallamy et al.,(2011)**[144] who found that serum vaspin levels were significantly correlated with the markers of insulin resistance and the glycemic indices suggesting that vaspin level may play a role in glucose metabolism and may be a part of the protective mechanisms aimed to reduce insulin resistance in humans.

# CONCLUSION

# CONCLUSION

The conclusions derived from the present study are:

- Vaspin, a visceral adipose tissue derived factor with potential antiprotease properties and insulin sensitising effects is increased in obesity.
- Vaspin levels are positively correlated and associated with the BMI, the measures of obesity and insulin resistance in the form of HOMA –IR.
- Vaspin could be used as a biomarker in obesity and as an indicator of cardiovascular risk.
- Vaspin also plays an important role in the pathogenesis of obesity and its related metabolic disorders.
- Hence Vaspin can also be used as a circulating biomarker for early identification of obesity related metabolic alterations and thus can enable us to make interventions at the earliest.

# SUMMARY

# SUMMARY

A study was conducted to determine the circulating Serum Vaspin levels in humans with obesity in order to assess its association and link to obesity related metabolic alterations.

Thirty obese subjects and thirty non obese subjects participated in the study.

The serum vaspin levels, the measures of obesity, the lipid profile and the insulin resistance was obtained, assessed and compared in both the study groups and the results showed a significant increase in the values of the parameters in the obese subjects than in the non obese subjects.

The serum vaspin levels were correlated with the above said parameters in obese subjects and a significant positive correlation was also obtained between them.

Thus the association and the link of vaspin to obesity and its related metabolic complication were established and can be used as a maker in obesity.

The study population in this study is relatively small and there are an unequal number of gender representatives. The correlation between the gender and the vaspin levels has not been made as there are more women subjects in my study.

Use of a larger sample size and an equal number of gender representatives and also gold standard methods as an indicator of obesity and insulin resistance would help get a clearer picture of the role of vaspin as a biomarker of obesity and its related metabolic alterations.

This would be my suggestion for future studies in this field.

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# ANNEXURES

# INSTITUTIONAL ETHICS COMMITTEE
# MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

## CERTIFICATE OF APPROVAL

To
Dr.Thamizh Valli.D.,
Post Graduate in M.D.Physiology
Institute of Physiology and Experimental Medicine
Madras Medical College
Chennai 600 003

Dear Dr.Thamizh Valli.D.,

The Institutional Ethics Committee has considered your request and approved your study titled **" ESTIMATION OF THE SERUM VASPIN LEVELS IN INDIVIDUALS WITH OBESITY AS A NOVEL CIRCULATING AND THERAPEUTIC BIOMARKER FOR OBESITY AND ITS RELATED METABOLIC ALTERATIONS" NO. 23062016.**

The following members of Ethics Committee were present in the meeting hold on **07.06.2016** conducted at Madras Medical College, Chennai 3

| | |
|---|---|
| 1.Dr.C.Rajendran, MD., | :Chairperson |
| 2.Dr.Isaac Christian Moses,MD.Ph.D.Dean(FAC)MMC,Ch-3 | :Deputy Chairperson |
| 3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3 | :MemberSecretary |
| 4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3 | : Member |
| 5.Prof.P.Raghumani,MS, Prof. of Surgery,RGGGH,Ch-3 | : Member |
| 6.Prof.Baby Vasumathi, Director, Inst. of O&G,Ch-8 | : Member |
| 7.Prof.K.Ramadevi,MD, Director,Inst.of Bio-Chem,MMC,Ch-3 | : Member |
| 8.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3 | : Member |
| 9.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3 | : Lay Person |
| 10.Thiru S.Govindasamy, BA.,BL,High Court,Chennai | : Lawyer |
| 11.Tmt.Arnold Saulina, MA.,MSW., | :Social Scientist |

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

28/6/16

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

# தகவல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

**சென்னை மருத்துவ கல்லூரியில் உடற்பருமன் அதிகமான நோயாளியின் இரத்ததில் வாள்பின் அளவு கன்டறியும் ஓர் ஆராய்க்கி**

ஆய்வாளர் : த.தமிழ் வள்ளி
முதலாம் ஆண்டு பட்ட மேற்படிப்பு மாணவி.
உடல் இயங்கியல் துறை
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600 003.

மனிதர்களிடையே உடற் பருமன் அதிக அளவில் வளர்ந்து கொண்டு வருகின்றது. இதனால் ஏற்படும் அபாயம் என்னவென்றால் அதிக உடற்பருமன் உள்ள மதிதர்கள் கீழ்கண்ட வியாதிகளால் பாதிப்புக்கு உள்ளாவர்கள்.

உதாரணம் :
1. சர்க்கரை வியாதி
2. உயர் ரத்த அமுத்தம்
3. இருதயக் கோளாறு
4. கேன்சர்
5. கை, கால் முடக்கம்

உடற்பருமன் உள்ளவர்களை ஆய்வு செய்வதால் அவர்களிடையே உள்ள நோய் அறிகுறிகளை ஆரம்ப கால கட்டத்தில் கண்டு அறிந்து, அந்த வியாதிக்காண மருத்துகளை உட்கொண்டு தீர்வு பெறலாம்.

இந்த ஆய்வில் நமது மூன்றாம் நிலை மருத்துவமனையில் உடற்பருமன் ஏற்படுத்தும் பாதிப்புக்களை பற்றி ஆராயப்படுகிறது.

இந்த ஆய்வில் ஆகும் அதிகப்படியான செலவிற்கு நோயாளிகளிடமிருந்து பணம் பெற்றுக் கொள்ளப்படமாட்டாது.

இந்த ஆய்வின் முடிவுகள் இறுதியில் பரிசுரிக்கப்படும் இந்த ஆய்வை பற்றிய சந்தேகங்கள் முழுமையாக தங்களுக்கு விளக்கப்படும், தொடர்பு கொள்ள வேண்டியவர், த.தமிழ்வள்ளி - 9381422260.

ஆய்வாளர் கையொப்பம்.

பங்கேற்பாளர் / பாதுகாவலர்

கையொப்பம் / இடதுகை பெருவுரல் ரேகை

தேதி :

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினாடளே, அரது நிறுவனத்திடமோ தேவைபட்டால் பகுர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வில் முடிவுகளை வெளியிடும் போது எனது பெயரோ, அடையாளமோ வெளியிடப்படமாட்டாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்டேன். இந்த ஆய்விறகாக எனது இரத்தம் பரிசோதனை செய்துக் கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்று தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தில் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்றும் தெரிந்து கொண்டேன்.

ஆய்வாளர் கையொப்பம் : D. Thazhvalli

பங்கேற்பாளர் / பாதுகாவலர்

கையொப்பம் / இடதுகை பெருவிரல் ரேகை

# சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

**சென்னை மருத்துவ கல்லூரியில் உடற்பருமன் அதிகமான நோயாளியின் இரத்ததில் வாள்பின் அளவு கன்டறியும் ஓர் ஆராய்க்கி**

பெயர் : வயது : தேதி :

பங்கேற்பானர் எண் :

என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முறையாக மருத்துவரிடம் கேட்டு அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுய நினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு, நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் நான் பங்கு பெறுகிறேன். இந்த ஆராய்ச்சியில் இருந்து நான் எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

# INFORMED CONSENT FORM

**Title of the study: "Estimation of the serum Vaspin levels in humans with obesity as a novel circulating and therapeutic biomarker for obesity and its related metabolic alterations"**

**Name of the Participant:**
**Name of the Principal Investigator:** Dr. THAMIZH VALLI.D
**Name of the Institution:**
Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai – 3.

**Documentation of the informed consent:**

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in :

**"Estimation of the serum Vaspin levels in humans with obesity as a novel circulating and therapeutic biomarker for obesity and its related metabolic alterations"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past_____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
11. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
12. I have understood that my identity will be kept confidential if my data are publicly presented.
13. I have had my questions answered to my satisfaction.
14. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

**Name and signature / thumb impression of the participant (or legal representative if participant incompetent)**

Name _____ Signature_____

Date_____

**Name and Signature of the investigator or his representative obtaining consent:**

Name _____ Signature_____

Date_____

# PATIENT PROFORMA

**Name:**

**Age/ Sex:**

**Address:**

**OP No.:**

**Occupation:**

History of diabetes mellitus:

History of Hypertension:

History of associated illness:

a. Thyroid disorders & cancers
b. Renal & hepatic diseases
c. Swellings in & around the neck region

History of smoking and alcohol abuse:

History of taking chronic medications or hormonal therapies:

Investigations:

EXAMINATION:

**General Physical examination:**

**Anthropometric measurements:**

Ht:

Wt:

WC:

HC:

W/H Ratio:

**Vitals:**

Pulse rate:

Blood pressure:

Respiratory Rate:

Temperature:

**Systemic examination:**

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system

# MASTER CHARTS

# BASELINE CHARACTERISTICS – OBESE

| S.NO | AGE | BMI | Waist Circumference (cms) | Hip circumference ( Cms) | Waist/ Hip Ratio | FBS ( mg/dl) | Sr.Insulin (µIu/L) | HOMA-IR | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | TGL (mg/dl) | LDL/HDL Ratio | TC/HDL Ratio | VASPIN ( ng/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36 | 48 | 125 | 140 | 0.9 | 75 | 9.79 | 1.81 | 195 | 49 | 124 | 109 | 2.53 | 4 | 0.541 |
| 2 | 36 | 38 | 125 | 137 | 0.91 | 73 | 13.35 | 2.4 | 207 | 35 | 139 | 163 | 3.97 | 5.9 | 0.038 |
| 3 | 50 | 39 | 130 | 145 | 0.89 | 81 | 28.42 | 5.68 | 191 | 34 | 120 | 184 | 3.53 | 5.6 | 0.539 |
| 4 | 50 | 47.3 | 120 | 137 | 0.88 | 111 | 45.05 | 12.34 | 212 | 38 | 146 | 139 | 3.84 | 5.6 | 3.585 |
| 5 | 35 | 36 | 102 | 115 | 0.88 | 98 | 34.13 | 8.25 | 225 | 58 | 121 | 231 | 2.09 | 3.9 | 2.024 |
| 6 | 37 | 45 | 135 | 150 | 0.9 | 90 | 25.23 | 5.6 | 163 | 34 | 95 | 170 | 2.79 | 4.8 | 0.265 |
| 7 | 35 | 37 | 104 | 115 | 0.9 | 88 | 28.34 | 6.15 | 231 | 39 | 163 | 143 | 4.18 | 5.9 | 1.639 |
| 8 | 45 | 39.2 | 128 | 140 | 0.91 | 66 | 101.7 | 16.5 | 168 | 30 | 108 | 151 | 3.60 | 5.6 | 1.122 |
| 9 | 50 | 42.1 | 119 | 135 | 0.88 | 118 | 22.01 | 6.41 | 185 | 38 | 118 | 146 | 3.11 | 4.9 | 0.867 |
| 10 | 50 | 45 | 130 | 145 | 0.89 | 95 | 38.17 | 8.95 | 228 | 45 | 164 | 94 | 3.64 | 5.1 | 1.453 |
| 11 | 36 | 42 | 130 | 140 | 0.92 | 100 | 26.05 | 6.43 | 221 | 42 | 164 | 75 | 3.90 | 5.3 | 0.216 |
| 12 | 50 | 38 | 113 | 125 | 0.9 | 95 | 14.8 | 3.47 | 171 | 44 | 101 | 129 | 2.30 | 3.9 | 1.894 |
| 13 | 50 | 37 | 116 | 120 | 9.96 | 87 | 19.54 | 4.19 | 199 | 59 | 119 | 104 | 2.02 | 3.4 | 0.779 |
| 14 | 45 | 51 | 119 | 134 | 0.88 | 87 | 10.18 | 2.18 | 346 | 64 | 252 | 148 | 3.94 | 5.4 | 2.975 |
| 15 | 50 | 45 | 121 | 135 | 0.89 | 138 | 19.6 | 6.67 | 270 | 27 | 194 | 243 | 7.19 | 10 | 1.487 |

| S.NO | AGE | BMI | Waist Circumference (cms) | Hip circumference ( Cms) | Waist/ Hip Ratio | FBS ( mg/dl) | Sr.Insulin (µIu/L) | HOMA-IR | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | TGL (mg/dl) | LDL/HDL Ratio | TC/HDL Ratio | VASPIN ( ng/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 45 | 37 | 102 | 115 | 0.88 | 92 | 11.52 | 2.61 | 220 | 37 | 157 | 132 | 4.24 | 5.9 | 2.397 |
| 17 | 40 | 40 | 112 | 125 | 0.89 | 86 | 27.49 | 5.83 | 161 | 29 | 93 | 194 | 3.21 | 5.6 | 1.184 |
| 18 | 37 | 42 | 112 | 120 | 0.93 | 69 | 29.65 | 5.05 | 243 | 22 | 176 | 225 | 8.00 | 11 | 0.072 |
| 19 | 35 | 38 | 110 | 125 | 0.88 | 76 | 13.28 | 2.49 | 182 | 35 | 119 | 138 | 3.40 | 5.2 | 3.585 |
| 20 | 40 | 38 | 115 | 130 | 0.88 | 101 | 20.2 | 5.03 | 168 | 41 | 104 | 117 | 2.54 | 4.1 | 3.585 |
| 21 | 45 | 40 | 109 | 120 | 0.9 | 95 | 17.61 | 4.13 | 144 | 39 | 83 | 110 | 2.13 | 3.7 | 0.319 |
| 22 | 35 | 41 | 116 | 130 | 0.89 | 72 | 19.27 | 3.42 | 148 | 38 | 88 | 108 | 2.32 | 3.9 | 0.065 |
| 23 | 40 | 37 | 105 | 115 | 0.91 | 118 | 28.77 | 8.38 | 146 | 35 | 87 | 120 | 2.49 | 4.2 | 0.2 |
| 24 | 45 | 42 | 104 | 117 | 0.88 | 86 | 13.52 | 2.87 | 156 | 54 | 82 | 100 | 1.52 | 2.9 | 0.469 |
| 25 | 40 | 46 | 110 | 124 | 0.88 | 92 | 31.23 | 7.09 | 157 | 48 | 87 | 108 | 1.81 | 3.3 | 1.108 |
| 26 | 50 | 38 | 110 | 115 | 0.95 | 44 | 11.5 | 1.24 | 127 | 42 | 70 | 74 | 1.67 | 3 | 0.537 |
| 27 | 35 | 38 | 110 | 115 | 0.95 | 79 | 29.09 | 5.67 | 205 | 35 | 35 | 122 | 1.00 | 5.9 | 0.664 |
| 28 | 35 | 37 | 111 | 120 | 0.92 | 102 | 62.3 | 15.69 | 106 | 37 | 47 | 109 | 1.27 | 2.9 | 3.585 |
| 29 | 35 | 37 | 111 | 120 | 0.92 | 102 | 62.3 | 15.69 | 106 | 37 | 47 | 109 | 1.27 | 2.9 | 0.145 |
| 30 | 35 | 37 | 111 | 120 | 0.92 | 102 | 62.3 | 15.69 | 106 | 37 | 47 | 109 | 1.27 | 2.9 | 0.319 |

# BASELINE CHARACTERISTICS – NON OBESE

| S.NO | AGE | BMI | Waist Circumference (cms) | Hip circumference ( Cms) | Waist/ Hip Ratio | FBS ( mg/dl) | Sr.Insulin (µIu/L) | HOMA-IR | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | TGL (mg/dl) | LDL/HDL Ratio | TC/HDL Ratio | VASPIN ( ng/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 35 | 24 | 82 | 102 | 0.8 | 37 | 12.07 | 1.10 | 128 | 26 | 77 | 123 | 3.0 | 4.9 | 0.361 |
| 2 | 30 | 24 | 82 | 105 | 0.78 | 50 | 5.63 | 0.70 | 219 | 24 | 108 | 435 | 4.5 | 9.1 | 0.689 |
| 3 | 35 | 20 | 83 | 105 | 0.79 | 73 | 4.52 | 0.81 | 145 | 43 | 82 | 98 | 1.9 | 3.4 | 1.592 |
| 4 | 35 | 21 | 83 | 107 | 0.77 | 67 | 8.87 | 1.47 | 175 | 44 | 110 | 105 | 2.5 | 4 | 0.921 |
| 5 | 36 | 24 | 82 | 105 | 0.78 | 66 | 10.27 | 1.67 | 178 | 32 | 127 | 94 | 4.0 | 5.6 | 0.532 |
| 6 | 37 | 22 | 83 | 105 | 0.79 | 61 | 6.54 | 0.99 | 147 | 47 | 77 | 117 | 1.6 | 3.1 | .42 |
| 7 | 37 | 24 | 84 | 105 | 0.8 | 94 | 37.7 | 8.75 | 109 | 32 | 63 | 69 | 2.0 | 3.4 | 0.386 |
| 8 | 38 | 24 | 84 | 107 | 0.78 | 63 | 15.63 | 2.43 | 194 | 30 | 127 | 185 | 4.2 | 6.5 | 0.301 |
| 9 | 35 | 21 | 83 | 110 | 0.75 | 57 | 10.96 | 1.54 | 153 | 28 | 107 | 91 | 3.8 | 5.5 | 0.642 |
| 10 | 36 | 23 | 81 | 110 | 0.73 | 71 | 6.87 | 1.20 | 116 | 39 | 64 | 64 | 1.6 | 3 | 0.71 |
| 11 | 36 | 24 | 82 | 100 | 0.82 | 56 | 7.29 | 1.01 | 158 | 41 | 99 | 90 | 2.4 | 3.9 | 0.71 |
| 12 | 39 | 24 | 82 | 100 | 0.82 | 73 | 10.92 | 1.97 | 203 | 56 | 128 | 94 | 2.3 | 3.6 | 1.323 |
| 13 | 40 | 22 | 82 | 110 | 0.75 | 67 | 13.44 | 2.22 | 133 | 42 | 75 | 78 | 1.8 | 3.2 | 0.529 |
| 14 | 35 | 18 | 83 | 100 | 0.83 | 66 | 12.03 | 1.96 | 177 | 49 | 108 | 100 | 2.2 | 3.6 | 0.881 |
| 15 | 37 | 20 | 84 | 100 | 0.84 | 68 | 7.69 | 1.29 | 157 | 44 | 101 | 59 | 2.3 | 3.6 | 1.662 |

| S.NO | AGE | BMI | Waist Circumference (cms) | Hip circumference ( Cms) | Waist/ Hip Ratio | FBS ( mg/dl) | Sr.Insulin (µIu/L) | HOMA-IR | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | TGL (mg/dl) | LDL/HDL Ratio | TC/HDL Ratio | VASPIN ( ng/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 37 | 25 | 84 | 100 | 0.84 | 80 | 10.07 | 1.99 | 167 | 42 | 100 | 123 | 2.4 | 4 | 0.058 |
| 17 | 35 | 25 | 83 | 105 | 0.79 | 74 | 11.59 | 2.12 | 169 | 28 | 93 | 238 | 3.3 | 6 | 1.509 |
| 18 | 35 | 25 | 83 | 105 | 0.79 | 64 | 7.25 | 1.15 | 201 | 31 | 141 | 146 | 4.5 | 6.5 | 0.693 |
| 19 | 37 | 25 | 82 | 102 | 0.8 | 53 | 8.14 | 1.07 | 221 | 52 | 139 | 151 | 2.7 | 4.3 | 1.167 |
| 20 | 38 | 21 | 82 | 102 | 0.8 | 53 | 13.37 | 1.75 | 151 | 40 | 91 | 101 | 2.3 | 3.8 | 0.258 |
| 21 | 40 | 22 | 82 | 102 | 0.8 | 39 | 4.34 | 0.42 | 245 | 63 | 155 | 134 | 2.5 | 3.9 | 0.693 |
| 22 | 37 | 25 | 83 | 105 | 0.79 | 53 | 9.26 | 1.21 | 133 | 45 | 64 | 122 | 1.4 | 3 | 0.129 |
| 23 | 35 | 21 | 83 | 105 | 0.79 | 17 | 10.22 | 0.43 | 127 | 36 | 65 | 131 | 1.8 | 3.5 | 0.012 |
| 24 | 50 | 24 | 86 | 109 | 0.78 | 55 | 14.47 | 1.97 | 142 | 33 | 75 | 171 | 2.3 | 4.3 | 0.71 |
| 25 | 39 | 21 | 86 | 109 | 0.78 | 54 | 8.89 | 1.19 | 186 | 43 | 117 | 129 | 2.7 | 4.3 | 0.471 |
| 26 | 38 | 24 | 87 | 109 | 0.79 | 62 | 5.72 | 0.88 | 130 | 42 | 72 | 82 | 1.7 | 3.1 | 1.623 |
| 27 | 35 | 23 | 82 | 100 | 0.82 | 60 | 10.77 | 1.60 | 155 | 32 | 108 | 76 | 3.4 | 4.8 | 1.911 |
| 28 | 37 | 24 | 82 | 100 | 0.82 | 64 | 4.91 | 0.78 | 159 | 34 | 108 | 86 | 3.2 | 4.7 | 3.97 |
| 29 | 39 | 23 | 86 | 107 | 0.8 | 87 | 20.68 | 4.44 | 163 | 33 | 104 | 130 | 3.2 | 4.9 | 0.027 |
| 30 | 37 | 23 | 86 | 107 | 0.8 | 78 | 21.28 | 4.10 | 217 | 51 | 134 | 160 | 2.6 | 4.3 | 0.13 |

## KEY TO MASTER CHART

BMI – Body Mass Index

FBS – Fasting Blood Sugar

HOMA – IR – Homeostatic model assessment- Insulin resistance

TC – Total Cholesterol

HDL – High density lipoprotein

TGL – Triglycerides

LDL – Low Density Lipoprotein